Use of Inducible Disaccharidases To Assess the Importance of Different Carbohydrate Sources for Bacteroides ovatus Growing in the Intestinal Tracts of Germfree Mice

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Patterns of disaccharidase expression were used to determine which polysaccharides were the major sources of carbohydrate for Bacteroides ovatus growing in the intestinal tracts of monocolonized germfree mice. Results indicate that B. ovatus grows on a variety of different carbohydrates, which are present in low concentrations, rather than relying on one type of carbohydrate as the major carbohydrate source.

Bacteroides ovatus, a species of gram-negative obligate anaerobes found in the human colon, can utilize a variety of polysaccharides (5). Presumably, the ability to obtain carbon and energy from polysaccharides contributes to the survival of B. ovatus in the intestinal tract because most of the carbohydrate that reaches the colon is in the form of polysaccharides. The versatility of B. ovatus raises the question of whether it acts as a scavenger in the intestine, utilizing small amounts of many different polysaccharides, or whether it preferentially utilizes abundant polysaccharides such as xylan. Previous studies, in which mixtures of polysaccharide utilization mutants of B. ovatus and the wild type were used to colonize the intestinal tracts of germfree mice, had indicated that α-galactosidases might be important substrates for B. ovatus growing in the intestine (6, 7). Mutants deficient in the ability to utilize the galactomannan guar gum were unable to compete with the wild type in the germfree mice (6). A mutant of B. ovatus in which an α-galactosidase gene (α-galactosidase III) was disrupted was also unable to compete with the wild type (7). However, the role of α-galactosidase III was not clear. It had no role in galactomannan utilization, nor was its expression induced by any of the polysaccharides tested (7). A possible explanation of the deleterious effect of this mutation was that the inducing α-galactoside substrate was not commercially available but was abundant in the chow diet fed to the mice.

To assess which polysaccharides were important substrates for B. ovatus growing in the intestines of monocolonized germfree mice, we took advantage of the fact that disaccharidases of B. ovatus are generally expressed at high levels only when bacteria are growing on the inducing substrate. Thus, disaccharidase expression patterns of B. ovatus taken directly from the ceca of monocolonized mice should indicate which polysaccharides were being utilized in the intestine. Although α-galactosidase expression patterns of B. ovatus had been determined previously (1, 7), expression patterns for other disaccharidases had not been established. Disaccharidase activities in extracts of B. ovatus growing on different carbohydrates were determined as described previously for α-galactosidase (1, 7). Conditions used to assay α-galactosidases proved to be optimal for the other disaccharidases as well (data not shown). Results are shown in Table 1. Each value is an average of values from at least three separate experiments in which duplicate determinations were made. In several cases, the same type of disaccharidase activity was induced by more than one polysaccharide. pI values were determined as described previously for α-galactosidases (7). In most cases, the same activity induced during growth on different substrates had the same pI value (Table 1). As can be seen from Table 1, most of the polysaccharides and disaccharidases tested were associated with a distinct pattern of disaccharidase activities.

It is interesting that some of the disaccharidase activities produced during growth on some polysaccharides (Table 1) were not necessarily the ones expected from the structure of the polysaccharide. For example, since xylan contains α-linked arabinose branches and a β-linked xylose backbone, elevated expression of α-arabinosidase and β-xylosidase activities would be expected. However, in addition to these activities, there was also a relatively high level of α-galactosidase activity in the xylan-grown cells. The pI of this activity suggested that it was due to α-galactosidase II, an enzyme whose synthesis was induced by melibiose, raffinose, and stachyose (1). Similarly, whereas most of the sugar residues in hog gastric mucin are β linked, the highest disaccharidase activities in B. ovatus grown on mucin were those of α-glucosidase and α-galactosidase (Table 1). The expression pattern associated with hog gastric mucin might be explained by the fact that growth of B. ovatus on this substrate was poor. Thus, B. ovatus could have been utilizing some minor component of the mucin mixture.

Germinfree mice were colonized with B. ovatus 0038 as described previously (3, 4). Three separate sets of mice (three per set in separate isolators) were used. B. ovatus grew to high concentrations (10^10 to 3 x 10^10/g [wet weight]) in the ceca of the germfree animals. Ceca and contents from three mice were pooled and homogenized in 50 mM potassium phosphate buffer (pH 7.3) at 4°C, and a bacterial fraction was obtained by differential centrifugation as described previously for Bacteroides thetaiotaomicron (3, 4). Bacteria in this fraction were disrupted by sonication, and the cell extracts were assayed for the disaccharidases listed in Table 1. pI values of the activities were also determined. Results are shown in Table 2.

The highest activities in the bacterial fraction taken directly from the ceca of the colonized mice were β-glucosidase, α-glucosidase, and α-galactosidase (Table 2). These
TABLE 1. Specific activities of disaccharidases in cell extracts from B. ovatus grown on different carbohydrates

<table>
<thead>
<tr>
<th>Carbohydrate in medium</th>
<th>β-Glc&lt;sup&gt;a&lt;/sup&gt;</th>
<th>β-Gal&lt;sup&gt;b&lt;/sup&gt;</th>
<th>α-Glc&lt;sup&gt;c&lt;/sup&gt;</th>
<th>α-Gal</th>
<th>β-Xyl</th>
<th>α-Ara&lt;sup&gt;d&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mannose</td>
<td>—</td>
<td>—</td>
<td>0.1</td>
<td>0.1</td>
<td></td>
<td>0.1</td>
</tr>
<tr>
<td>Galactose</td>
<td>—</td>
<td>0.1</td>
<td>1.0</td>
<td></td>
<td></td>
<td>2.5</td>
</tr>
<tr>
<td>Lactose</td>
<td>0.1</td>
<td>1.0</td>
<td>0.1</td>
<td>0.3</td>
<td></td>
<td>4.4</td>
</tr>
<tr>
<td>Maltose</td>
<td>0.1</td>
<td>0.2</td>
<td>2.5</td>
<td>0.1</td>
<td>1.3</td>
<td>1.0</td>
</tr>
<tr>
<td>Raffinose</td>
<td>—</td>
<td>0.1</td>
<td>—</td>
<td>0.1</td>
<td></td>
<td>0.5</td>
</tr>
<tr>
<td>Guar gum</td>
<td>—</td>
<td>0.2</td>
<td>—</td>
<td>0.1</td>
<td></td>
<td>0.4</td>
</tr>
<tr>
<td>Arabinogalactan</td>
<td>0.1</td>
<td>0.3</td>
<td>0.3</td>
<td>0.7</td>
<td>0.4</td>
<td>0.7</td>
</tr>
<tr>
<td>Galactomannan</td>
<td>0.2</td>
<td>0.2</td>
<td>1.7</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup> Defined medium (1) with the indicated carbohydrate as the only carbon source.

<sup>b</sup> Abbreviations: β-Glc, β-glucosidase; β-Gal, β-galactosidase; α-Glc, α-glucosidase; α-Gal, α-galactosidase; β-Xyl, β-xylanidase; α-Ara, α-arabinosidase.

Some of the values shown here for α-galactosidase have been published previously (7) but were included for completeness. 1 U = 0.1 μmol of p-nitrophenol per min at 37°C.

<sup>c</sup> In all cases, the pl of the activity was 5.1.

<sup>d</sup> In all cases, the pl of the activity was 5.4.

<sup>e</sup> In both cases, the pl of the activity was 8.2.

<sup>f</sup> Specific activity of less than 0.1 U/mg of protein.

The results in Table 2 rule out most of the substrates shown in Table 1 as sole sources of carbohydrate for B. ovatus growing in the intestinal tracts of mice fed a chow diet. For example, if xylan or arabinogalactan were major substrates, xylanidase or arabinosidase should have been detectable. The failure to see evidence of xylan utilization is surprising because xylan should be one of the most abundant polysaccharides in the chow diet. However, it should be kept in mind that the carbohydrates that are most likely to be used by bacteria are the most fermentable, one of polysaccharides that is insoluble may be ignored by bacteria in favor of a much less abundant one that is soluble and thus more easily degraded.

Since mice are lactase deficient and lactose from whey in the chow should also reach the cecum. However, lactose appeared not to be a major carbohydrate source, because β-galactosidase levels were barely detectable. If raffinose or stachyose was the main carbohydrate source, no α-glucosidase or β-glucosidase should have been detected. Even the expression pattern of bacteria grown on galactose did not correspond exactly to the pattern of expression in bacteria taken directly from colonized mice.

The most likely explanation of the results shown in Table 2 is that they reflect low-level utilization of many different substrates. Previous studies done with pure cultures of B. thetaotaomicron growing in laboratory media indicated that the level of expression of polysaccharidases was little affected by the growth rate but was affected considerably by the substrate level (2, 3). Low levels of inducing substrate led to low-level expression of the enzyme. Thus, utilization of a number of different polysaccharides, which are present only in low concentrations, would be expected to give an expression pattern similar to that seen in Table 2.

The fact that we detected only trace amounts of α-galactosidase (1, pl, 5.6), the α-galactosidase induced during growth on the galactomannan guar gum, indicated that guar gum is not a major source of carbohydrate for B. ovatus growing in the mice. This result, taken together with our earlier finding that mutants of B. ovatus which were unable to grow on guar gum were deficient in colonization of germfree mice, suggests that loss of even a minor substrate...
can be deleterious in vivo. We failed to detect even trace amounts of α-galactosidase III in extracts from the cecal bacterial fraction despite the fact that a mutation which disrupted this gene was deleterious in the germfree mouse model. Thus, our original hypothesis that chow diet contains an inducer of this enzyme is not correct, and the deleterious effect of this mutation remains unexplained.

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REFERENCES