Growth of various intestinal bacteria on alternansucrase-derived oligosaccharides

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ABSTRACT

Aims: To determine whether alternansucrase (ASR)-derived oligosaccharides can support the in vitro growth of various intestinal bacteria.

Methods and Results: Growth was assessed from each culture after incubation in a medium containing ASR-derived oligosaccharide as sole carbohydrate source. Most of the Bifidobacterium spp. tested showed growth on all five of the oligosaccharides tested while the Lactobacillus spp., Bacteroides thetaiotaomicron, coliforms and pathogenic bacteria displayed no or little growth.

Conclusions: The ASR-derived oligosaccharides were selectively utilized by many of the Bifidobacterium spp. tested but did not support significant growth of the Lactobacillus spp., Bact. thetaiotaomicron, coliforms and pathogenic bacteria tested.

Significance and Impact of the Study: Alternansucrase-derived oligosaccharides are a potential source of new prebiotics.

Keywords: alternansucrase, Bifidobacterium, glucooligosaccharides, oligosaccharides, prebiotic.

INTRODUCTION
Prebiotics are dietary supplements used to selectively stimulate the growth of beneficial intestinal microbiota in humans and animals. The proliferation and maintenance of beneficial intestinal bacteria may provide health-promoting factors that suppress disease (Playne 1995; Reid 1999). Beneficial intestinal bacteria have included both Bifidobacterium and Lactobacillus spp. (Mitsuoka 1992; Salminen et al. 1998). In addition to stimulating growth of beneficial bacteria, prebiotics should not support significant growth of undesirable intestinal species such as coliform or pathogenic bacteria (Gibson and Roberfroid 1995). Prebiotics are usually carbohydrates and have included fructooligosaccharides (FOS) and glucooligosaccharides (GOS) (Monsan and Paul 1995; Crittenden and Playne 1996). One promising source of GOS is through enzymatic synthesis using bacterial glucansucrases. Glucansucrases can synthesize short-chain oligosaccharides containing a variety of linkages when chain terminating acceptor molecules are added to the enzymatic reaction (Monsan and Paul 1995; Côté et al. 2003). An attractive feature of glucansucrase-derived oligosaccharides is that, a variety of potential prebiotics can be synthesized compared with FOS preparations. Because of the complex nature of intestinal microbial ecosystems, no single prebiotic preparation may be suitable for all applications or individuals. Alternansucrase (ASR) from Leuconostoc mesenteroides has been used to synthesize oligosaccharides using acceptor molecules (Côté et al. 2003). The prebiotic properties of ASR-derived oligosaccharides, however, have never been reported. The goal of this study was to determine if ASR-oligosaccharides could stimulate in vitro growth of pure cultures of colonic bacteria.
MATERIALS AND METHODS

Oligosaccharides

The five oligosaccharide preparations used in this study were prepared from ASR acceptor reactions for a previous study (Côté et al. 2003). Each oligosaccharide was prepared from an acceptor carbohydrate, sucrose and the bacterial enzyme ASR. ASR catalyses the synthesis of a large molecular mass α-glucan polymer from sucrose called alteman (Côté 2002). ASR can also synthesize low mass oligosaccharides instead of the high molecular weight native α-glucan polymer when certain chain-ending acceptor molecules are added to the reaction (Côté et al. 2003). Many chain-ending acceptor molecules have been tested for oligosaccharide synthesis using ASR and include carbohydrates such as maltose, melibiose, maltitol, raffinose and gentiobiose (Côté et al. 2003). The acceptor molecules used for oligosaccharide synthesis and the structure and composition of each oligosaccharide preparation used in this study is listed in Table 1.

Bacterial strains and media

Bacterial strains were obtained from ATCC (Manassas, VA, USA) (Table 2). Bacteroides thetaiotaomicron and Bifidobacterium spp. were grown on DSM 58 medium (Marx et al. 2000), Clostridium perfringens was grown on a modified TGY medium (Ionesco et al. 1976), Enterobacter aerogenes, Escherichia coli and Salmonella choleraesuis were grown on a modified defined medium (Edberg and Edberg 1988), and Lactobacillus species were grown on MRS medium (DeMan et al. 1960).

Growth determination

Test carbohydrates were added independently to each medium (0.5% w/v) as the sole carbohydrate source. Test media were filter sterilized (0.22 μm, Fisherbrand; Fisher Scientific, Pittsburgh, PA, USA) and added to sterilized 16-mm screw-cap test tubes to a volume of 9.0 ml. Oxygen was removed by adding Oxyrase to each medium (Oxyrase, Inc., Mansfield, OH, USA). Inoculum (0.25 ml) for each test medium was prepared by cultivating each microorganism for 24-48 h in the appropriate medium containing 0.5% glucose as the sole carbohydrate source. Cultures were incubated for 5 d at 37°C in an anaerobic jar using the GasPak system (Becton Dickinson Microbiology Systems, Sparks, MD, USA). Test media containing no carbohydrate were inoculated with each microorganism as a control to account for glucose carry-over. Growth was determined by subtracting A660 values (DU-64 Spectrophotometer; Beckman, Schaumburg, IL, USA) of control medium from each test media. Growth tests were performed in triplicate and A660 was reported as the mean ± SD.

Growth curves

Growth curves for Bifidobacterium adolescentis and Bifidobacterium pseudocatenulatum were determined for the two oligosaccharide preparations that displayed the highest A-values from growth determination tests. Media were prepared as described previously except that Hungate type anaerobic culture tubes (screw cap with 9-mm opening and butyl rubber stopper; Belco Glass, Inc., Vinland, NJ, USA)

Table 1 Composition of ASR-derived oligosaccharides used in this study (Côté et al. 2003)

<table>
<thead>
<tr>
<th>Acceptor</th>
<th>Acceptor product composition and structure</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gentiobiose</td>
<td>84% DP3, α-D-Glc-(1→6)β-D-Glc-(1→6)-D-Glc</td>
</tr>
<tr>
<td></td>
<td>9% DP4, [α-D-Glc-(1→6),β-D-Glc-(1→6)-D-Glc</td>
</tr>
<tr>
<td></td>
<td>7% DP4, α-D-Glc-(1→3)α-D-Glc-(1→6)-β-D-Glc-(1→6)-D-Glc</td>
</tr>
<tr>
<td>Maltitol</td>
<td>50% DP3, α-D-Glc-(1→6)α-D-Glc-(1→4)α-D-Glucitol (panitol)</td>
</tr>
<tr>
<td></td>
<td>25% DP2, α-D-Glc-(1→4)α-D-Glucitol (maltitol)</td>
</tr>
<tr>
<td></td>
<td>13% DP4, α-D-Glc-(1→6) panitol</td>
</tr>
<tr>
<td></td>
<td>12% DP4, α-D-Glc-(1→3) panitol</td>
</tr>
<tr>
<td>Maltose</td>
<td>67% DP3, 6º-O-α-D-glucosylmaltose (panose)</td>
</tr>
<tr>
<td></td>
<td>20% DP4, α-D-Glc-(1→3)α-D-Glc-(1→6)-α-D-Glucitol (1→4)-D-Glc</td>
</tr>
<tr>
<td></td>
<td>α-D-Glc-(1→6)-α-D-Glc-(1→4)-α-D-Glc-(1→4)-D-Glc</td>
</tr>
<tr>
<td></td>
<td>13% DP &gt; 4, structures not determined</td>
</tr>
<tr>
<td>Melibiose</td>
<td>90% DP3, α-D-Glc-(1→3)α-D-Gal-(1→6)-D-Glc</td>
</tr>
<tr>
<td></td>
<td>&lt;5% DP2, α-D-Gal-(1→6)-D-Glc (melibiose)</td>
</tr>
<tr>
<td></td>
<td>&lt;5% DP3, α-D-Glc-(1→4)α-D-Gal-(1→6)-D-Glc</td>
</tr>
<tr>
<td></td>
<td>&lt;5% DP4, structure not determined</td>
</tr>
<tr>
<td>Raffinose</td>
<td>85% DP4, α-D-Glc-(1→4)α-D-Gal-(1→6)-α-D-Glc-(1→4)β-D-Fru</td>
</tr>
<tr>
<td></td>
<td>10% DP4, α-D-Glc-(1→3)α-D-Gal-(1→6)-α-D-Glc-(1→4)β-D-Fru</td>
</tr>
<tr>
<td></td>
<td>&lt;5% DP3, α-D-Gal-(1→6)-α-D-Glc-(1→4)β-D-Fru (raffinose)</td>
</tr>
<tr>
<td></td>
<td>&lt;5% DP5 and higher, structures not determined</td>
</tr>
</tbody>
</table>
Table 2: Growth of various colonic bacteria on alternansucrase-derived oligosaccharides

<table>
<thead>
<tr>
<th>Organism tested and ATCC number</th>
<th>Glucose product</th>
<th>Gentiobiose product</th>
<th>Maltitol product</th>
<th>Melibiose product</th>
<th>Raffinose product</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bifidobacterium adolescentis ATCC 15703&lt;sup&gt;T&lt;/sup&gt;</td>
<td>0.99 ± 0.09</td>
<td>0.85 ± 0.03</td>
<td>0.90 ± 0.00</td>
<td>0.92 ± 0.04</td>
<td>0.85 ± 0.16</td>
</tr>
<tr>
<td>Bifidobacterium adolescentis ATCC 15704</td>
<td>0.82 ± 0.02</td>
<td>0.82 ± 0.01</td>
<td>0.71 ± 0.02</td>
<td>0.43 ± 0.00</td>
<td>0.23 ± 0.01</td>
</tr>
<tr>
<td>Bifidobacterium adolescentis ATCC 15705</td>
<td>0.94 ± 0.02</td>
<td>1.11 ± 0.01</td>
<td>0.95 ± 0.00</td>
<td>0.54 ± 0.12</td>
<td>0.24 ± 0.00</td>
</tr>
<tr>
<td>Bifidobacterium adolescentis ATCC 15706</td>
<td>0.95 ± 0.01</td>
<td>1.08 ± 0.01</td>
<td>0.06 ± 0.03</td>
<td>0.31 ± 0.04</td>
<td>0.19 ± 0.00</td>
</tr>
<tr>
<td>Bifidobacterium bifidum ATCC 29521&lt;sup&gt;T&lt;/sup&gt;</td>
<td>0.93 ± 0.00</td>
<td>0.04 ± 0.04</td>
<td>0.02 ± 0.00</td>
<td>0.02 ± 0.00</td>
<td>0.04 ± 0.00</td>
</tr>
<tr>
<td>Bifidobacterium boum ATCC 27917&lt;sup&gt;T&lt;/sup&gt;</td>
<td>0.71 ± 0.00</td>
<td>0.50 ± 0.00</td>
<td>0.39 ± 0.02</td>
<td>0.39 ± 0.00</td>
<td>0.18 ± 0.00</td>
</tr>
<tr>
<td>Bifidobacterium breve ATCC 15698&lt;sup&gt;T&lt;/sup&gt;</td>
<td>0.7 ± 0.00</td>
<td>0.46 ± 0.02</td>
<td>0.51 ± 0.00</td>
<td>0.37 ± 0.00</td>
<td>0.16 ± 0.00</td>
</tr>
<tr>
<td>Bifidobacterium catenulatum ATCC 27539&lt;sup&gt;T&lt;/sup&gt;</td>
<td>0.85 ± 0.00</td>
<td>0.84 ± 0.01</td>
<td>0.34 ± 0.01</td>
<td>0.60 ± 0.02</td>
<td>0.21 ± 0.02</td>
</tr>
<tr>
<td>Bifidobacterium gallicum ATCC 49850&lt;sup&gt;T&lt;/sup&gt;</td>
<td>0.90 ± 0.02</td>
<td>0.09 ± 0.01</td>
<td>0.13 ± 0.05</td>
<td>0.53 ± 0.00</td>
<td>0.00 ± 0.00</td>
</tr>
<tr>
<td>Bifidobacterium infantis ATCC 15697&lt;sup&gt;T&lt;/sup&gt;</td>
<td>0.8 ± 0.00</td>
<td>0.36 ± 0.00</td>
<td>0.69 ± 0.00</td>
<td>0.29 ± 0.02</td>
<td>0.25 ± 0.00</td>
</tr>
<tr>
<td>Bifidobacterium longum ATCC 15707&lt;sup&gt;T&lt;/sup&gt;</td>
<td>0.63 ± 0.00</td>
<td>0.15 ± 0.01</td>
<td>0.28 ± 0.00</td>
<td>0.65 ± 0.00</td>
<td>0.26 ± 0.01</td>
</tr>
<tr>
<td>Bifidobacterium pseudocatenulatum ATCC 27919&lt;sup&gt;T&lt;/sup&gt;</td>
<td>0.94 ± 0.07</td>
<td>0.91 ± 0.02</td>
<td>0.53 ± 0.03</td>
<td>0.81 ± 0.04</td>
<td>0.78 ± 0.02</td>
</tr>
<tr>
<td>Bifidobacterium ruminantium ATCC 49390&lt;sup&gt;T&lt;/sup&gt;</td>
<td>0.88 ± 0.00</td>
<td>0.04 ± 0.00</td>
<td>0.03 ± 0.01</td>
<td>0.79 ± 0.04</td>
<td>0.27 ± 0.00</td>
</tr>
<tr>
<td>Bifidobacterium thermophilum ATCC 28566</td>
<td>0.77 ± 0.00</td>
<td>0.46 ± 0.02</td>
<td>0.42 ± 0.01</td>
<td>0.51 ± 0.01</td>
<td>0.19 ± 0.00</td>
</tr>
<tr>
<td>Lactobacillus acidophilus ATCC 4356&lt;sup&gt;T&lt;/sup&gt;</td>
<td>0.86 ± 0.05</td>
<td>0.00 ± 0.00</td>
<td>0.04 ± 0.03</td>
<td>0.11 ± 0.00</td>
<td>0.01 ± 0.00</td>
</tr>
<tr>
<td>Lactobacillus casei ATCC 393&lt;sup&gt;T&lt;/sup&gt;</td>
<td>0.78 ± 0.07</td>
<td>0.57 ± 0.00</td>
<td>0.12 ± 0.01</td>
<td>0.13 ± 0.00</td>
<td>0.03 ± 0.02</td>
</tr>
<tr>
<td>Lactobacillus GG ATCC 53103</td>
<td>0.45 ± 0.00</td>
<td>0.00 ± 0.00</td>
<td>0.00 ± 0.00</td>
<td>0.00 ± 0.00</td>
<td>0.00 ± 0.00</td>
</tr>
<tr>
<td>Bacteroides thetaiotaomicron ATCC 29148&lt;sup&gt;T&lt;/sup&gt;</td>
<td>0.68 ± 0.07</td>
<td>0.16 ± 0.02</td>
<td>0.18 ± 0.01</td>
<td>0.2 ± 0.01</td>
<td>0.09 ± 0.01</td>
</tr>
<tr>
<td>Clostridium perfringens ATCC 13124&lt;sup&gt;T&lt;/sup&gt;</td>
<td>0.74 ± 0.05</td>
<td>0.00 ± 0.00</td>
<td>0.03 ± 0.00</td>
<td>0.02 ± 0.03</td>
<td>0.02 ± 0.00</td>
</tr>
<tr>
<td>Enterobacter aerogenes ATCC 35028</td>
<td>0.57 ± 0.06</td>
<td>0.02 ± 0.00</td>
<td>0.25 ± 0.02</td>
<td>0.0 ± 0.00</td>
<td>0.14 ± 0.00</td>
</tr>
<tr>
<td>Escherichia coli ATCC 8739</td>
<td>0.61 ± 0.02</td>
<td>0.00 ± 0.00</td>
<td>0.00 ± 0.00</td>
<td>0.00 ± 0.00</td>
<td>0.00 ± 0.00</td>
</tr>
<tr>
<td>Salmonella choleraesuis ATCC 14028&lt;sup&gt;*&lt;/sup&gt;</td>
<td>0.69 ± 0.00</td>
<td>0.00 ± 0.00</td>
<td>0.00 ± 0.00</td>
<td>0.00 ± 0.00</td>
<td>0.00 ± 0.00</td>
</tr>
</tbody>
</table>

<sup>T</sup> type strain.

Results are A<sub>660</sub> values determined in triplicate and reported as mean ± SD.

<sup>*</sup> Salmonella choleraesuis sp. choleraesuis serovar Typhimurium.

RESULTS

Growth on oligosaccharides

With the exception of Bifidobacterium bifidum ATCC 29521, most Bifidobacterium spp. tested displayed growth on the ASR-derived oligosaccharides (Table 2). The amount of growth displayed was variable and depended on the Bifidobacterium spp. or strain and the oligosaccharide. Bifidobacterium adolescentis ATCC 15703 and Bifidobacterium pseudocatenulatum displayed high growth on all of the oligosaccharides tested. Lactobacillus spp. tested displayed from very low to no growth on most of the oligosaccharides (Table 2).

Bacteroides thetaiotaomicron, C. perfringens and Ent. aerogenes displayed low or no growth on the oligosaccharides. Escherichia coli and S. choleraesuis did not display growth on any of the oligosaccharides tested (Table 2). Growth curves of Bif. adolescentis ATCC 15703 on the raffinose product or the maltitol product were nearly identical regardless of the inoculum used for each growth profile was pre-exposed to glucose or oligosaccharide (Fig. 1). Growth curves of Bif. pseudocatenulatum on the gentiobiose product were nearly identical regardless of whether the inoculum used was pre-exposed to glucose or gentiobiose product. (Fig. 2). Growth curves of Bif. pseudocatenulatum on the raffinose product, however, were different when the inoculum used for each profile was pre-exposed to glucose or raffinose product (Fig. 2). A much slower growth rate was exhibited on raffinose product when the inoculum was pre-exposed to glucose or oligosaccharide (Fig. 1).

DISCUSSION

The aim of this study was to determine if ASR-derived oligosaccharides could support the in vitro growth of Bifidobacterium spp., Lactobacillus spp. or Bact. thetaiotaomicron.
Bifidobacterium thermophilum is not a type strain but was used because it was isolated from bovine rumen (Scardovi et al. 1969). The ASR-derived oligosaccharides were selectively utilized by many of the Bifidobacterium spp. tested but did not support significant growth of the Lactobacillus spp., Bact. thetaiotaomicron, coliforms and pathogenic bacteria tested. In addition, Bif. adolescentis ATCC 15703 and Bif. pseudocatenulatum displayed high growth on all the oligosaccharides tested. Both species apparently have mechanisms that allow for utilization of a variety of oligosaccharides. Bifidobacterium spp. are saccharolytic anaerobes that reside in the mammalian gastrointestinal system, are generally considered beneficial for the host, and have been reported to use prebiotic oligosaccharides (Mitsuoka 1990). Schell et al. (2002) reported that the genome sequence of Bifidobacterium longum displayed a large number of predicted proteins specialized for oligosaccharide catabolism (glycosyl hydrolases and transporters), which supports phenotypic data concerning the saccharolytic capability of Bifidobacterium. Lactobacillus casei was the only Lactobacillus spp. to display growth on any of the oligosaccharides tested in this study. As reported in other studies, growth of Lactobacillus on FOS appears to be species or strain specific (Monsan and Paul 1995; Kaplan and Hutkins 2000). Bacteroides thetaiotaomicron, a saccharolytic procaryote (Holdeman et al. 1984), displayed low growth on most oligosaccharides tested in this study. Bacteroides thetaiotaomicron is a predominant procaryote in the human gastrointestinal system and has been reported to utilize a variety of oligosaccharides including FOS (Hidaka et al. 1986; Djouzi et al. 1995; Van Laere et al. 2000). However, Bact. thetaiotaomicron apparently lacks the enzymes or transporters needed to display significant growth on the oligosaccharide products used in this study.

ASR-oligosaccharides did not support significant growth of the coliforms (E. coli, Ent. aerogenes) or pathogens tested (C. perfringens and Salmonella). The ability of coliforms to utilize prebiotic oligosaccharides has been contradictory. Several studies have reported that FOS can support growth of E. coli, Enterobacter and Salmonella (Wang and Gibson 1993; Hartemink et al. 1997), while others have indicated no growth (Hidaka et al. 1986; Mitsuoka et al. 1987; Bailey et al. 1991; Monsan and Paul 1995). VanLaere et al. (2000) reported that arabinoooligosaccharides could support growth of E. coli but FOS could not. Clostridium perfringens is a saccharolytic and pathogenic organism (Sneath 1986) that possesses a wide variety glycosidic enzymes and transporters for carbohydrate utilization (Shimizu et al. 2002). It has been reported that C. perfringens cannot use FOS or GOS (Hidaka et al. 1986; Monsan and Paul 1995). In contrast, VanLaere et al. (2000) reported that a strain of C. perfringens fermented FOS. The coliforms and pathogens used in this study apparently lack specific mechanisms (hydrolases or transporters) for efficient use of ASR-derived oligosaccharides. Consequently, growth of a microorganism on a particular oligosaccharide may be strain specific (dependent on specific hydrolases and transporters) and dependent on growth conditions. Based on the growth curve data in this study, growth of Bif. adolescentis on the raffinose product or maltitol product did not require an adaptation period as the lag phases were nearly identical for both oligosaccharide products when compared with glucose. Efficient utilization of the raffinose product by Bif. pseudocatellulatum, however, may require a short adaptation period involving prior exposure to the oligosaccharide. This in vitro study has provided an initial assessment on the substrate specificities of some colonic bacteria on ASR-derived oligosaccharides. This study has also provided some evidence of a potential new source of prebiotic oligosaccharides for selective growth of certain Bifidobacterium spp. This study does not, however, allow a prediction of prebiotic effect in vivo as this would require additional studies involving mixed cultures (Wang and Gibson 1993) and human trials to account for the influence imposed by a complex faecal microbiota (Roberfroid 2001).

REFERENCES


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