Energy sources of major intestinal fermentative anaerobes$^{1,2}$

Abigail A. Salyers, Ph.D.

Bacteria account for approximately 30% of the volume of the contents of the human colon. The metabolic activities of these bacteria are undoubtedly a major factor in determining the chemical environment of the colon, and it is possible that colonic bacteria are involved in the etiology of diseases of the colon such as colon cancer (1). In recent years, a great deal of effort has been devoted to isolating and identifying the main components of the human colonic flora. Such studies have demonstrated that the colonic flora is extremely complex. It includes hundreds of distinct species, many of which had not been previously described (2-4). Attempts to determine whether the species composition of this flora depends on the host's diet have not yielded clear-cut answers. However, recent evidence appears to support the view that the species composition of the colonic flora is not significantly affected by diet (4-6).

Although diet seems to have little effect on the species composition of the human colonic flora, changes in diet may still produce considerable changes in the metabolic activities of the flora. For example, changes in diet will provide new types of substrates and this will lead to changes in the levels of different catabolic enzymes. At present, little is known about the possible range of the metabolic activities of the human colonic flora and about the effect of diet on these activities. One approach is to begin with the question: What are the natural energy sources for colon bacteria? If colon bacteria utilize dietary substances more readily than endogenously produced substances such as mucins, diet will have a much greater effect on the metabolic activities of the flora than would be the case if colon bacteria depend mainly on host-produced substances as sources of carbon and energy. The hypothesis that bacteria utilize mucins and other endogenous substances preferentially has been advanced to explain the stability of the flora (6). However, nutritional balance studies have shown that appreciable percentages of some dietary substances, such as plant cell wall polysaccharides, are not recovered in human feces (7). Since these substances are not degraded by mammalian enzymes, their disappearance is presumably due to digestion by intestinal bacteria. Thus, dietary substances are probably an important source of energy for at least some colon bacteria.

Utilization of polysaccharides by intestinal bacteria

Nearly all of the bacteria in the human colon are obligate anaerobes and most of them require a fermentable carbohydrate (2-4). Strong proteolysis is rare. Of the five major genera in the colon (Bacteroides, Eubacterium, Bifidobacterium, Peptostreptococcus, and Fusobacterium) only one genus, Fusobacterium, contains organisms that are predominantly nonsaccharolytic. The natural substrates of saccharolytic colon bacteria are probably complex carbohydrates. Simple sugars and disaccharides are absorbed efficiently from material passing through the small intestine and thus do not reach the colon. By contrast, most complex carbohydrates, e.g., plant cell wall polysaccharides and human mucins, are not digested in the human stomach or small intestine and are thus available to colon bacteria.

Some typical polysaccharide components of plant cell walls and human mucins are listed in Table 1. These polysaccharides are quite diverse with respect to composition and linkages. Moreover, they do not usually occur

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as isolated compounds, but rather as components of a complex matrix. Plant cell walls, for example, consist of an intricate network of polysaccharides, protein, and lignin, held together by covalent as well as noncovalent bonds (13). Similarly, mucins frequently occur as part of a complex, e.g., in ground substance or on the surface of epithelial cells. Thus, to demonstrate that a particular type of complex carbohydrate is utilized by colonic bacteria, it is necessary not only to establish that individual polysaccharide components can be utilized, but also that utilizable polysaccharides are actually accessible to bacterial attack in vivo.

Recent surveys of strains from 25 major species of colon bacteria have shown that many of these species are capable of fermenting individual noncellulosic plant cell wall polysaccharides and mucin polysaccharides in vitro (10, 11). Cellulolytic bacteria have also been isolated from human feces (12). Examples of bacterial species which degrade different types of polysaccharides are listed in Table 1. Most of these species fall into two genera, Bacteroides and Bifidobacterium. Some species, such as Bacteroides ovatus, Bacteroides thetaiotaomicron, and Bacteroides uniformis, are able to ferment a wide range of polysaccharides, representing a variety of components and linkages. The versatility of such organisms may aid their survival in the colon where they are confronted with a constantly changing mixture of different types of polysaccharides. These surveys are by no means exhaustive, but they do demonstrate that at least some colon bacteria can obtain carbon and energy from the types of polysaccharides that are found in plant cell walls or mucins.

The fact that individual polysaccharides are fermented by single strains of bacteria in vitro does not necessarily prove that polysaccharide utilization is significant in vivo. Many

**Table 1**
Source, composition, and bacterial utilization of some types of polysaccharide that are components of plant cell walls or mucins

<table>
<thead>
<tr>
<th>Polysaccharide</th>
<th>Composition*</th>
<th>Source</th>
<th>Bacterial species able to utilize the polysaccharide†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cellulose</td>
<td>Linear (\beta(1,4)) glucan (\beta(1,4))-linked xylose backbone, arabinose side chains</td>
<td>Plant cell walls</td>
<td>Bacteroides sp.</td>
</tr>
<tr>
<td>Xylan</td>
<td>Linear (\beta(1,3)) or mixed (\beta(1,3), \beta(1,4)) glucans</td>
<td>Plant cell walls</td>
<td>B. ovatus, Bacteroides eggerthii, B. fragilis subspecies a, B. vulgatus, Bifidobacterium adolescentis, B. infantis</td>
</tr>
<tr>
<td>Noncellulosic glucans (laminarin, lichenan)</td>
<td>Linear (\alpha(1,4)) linked polymers containing galacturonic acid (and esters)</td>
<td>Plant cell walls</td>
<td>B. thetaiotaomicron, B. uniformis, B. distasonis, B. &quot;T4-1&quot;</td>
</tr>
<tr>
<td>Pectins</td>
<td>Linear (\alpha(1,4)) linked polymers containing galacturonic acid (and esters)</td>
<td>Plant cell walls</td>
<td>B. thetaiotaomicron, B. ovatus, B. fragilis &quot;3452 A&quot;, B. vulgatus, Eubacterium eugen</td>
</tr>
<tr>
<td>Galactomannans (guar gum, locust bean gum)</td>
<td>(\beta(1,4))-linked mannose backbone with galactose side chains</td>
<td>Plant gums; food additives</td>
<td>B. ovatus, B. uniformis, Ruminococcus albus</td>
</tr>
<tr>
<td>Arabinogalactans</td>
<td>(\beta(1,4))-linked galactose backbone, arabinose side chains</td>
<td>Plant cell walls</td>
<td>B. thetaiotaomicron, B. ovatus, B. &quot;3452 A&quot;, B. uniformis, B. &quot;T4-1&quot;, B. fragilis &quot;3452 A&quot;, B. vulgatus, Eubacterium eugen</td>
</tr>
<tr>
<td>Mucopolysaccharides (chondroitin sulfate, heparin, hyaluronic acid)</td>
<td>Linear polymers containing hexosamine and uronic acid, sulfate esters</td>
<td>Sloughed epithelial cells, dietary meat</td>
<td>B. thetaiotaomicron, B. ovatus, B. &quot;3452 A&quot;, B. uniformis, B. &quot;T4-1&quot;, B. fragilis &quot;3452 A&quot;, B. vulgatus, Eubacterium eugen</td>
</tr>
<tr>
<td>Mucin glycoprotein</td>
<td>Polysaccharide (sialic acid, (\alpha)-fucose, hexose, hexosamine), protein</td>
<td>Swallowed saliva, goblet cell mucin</td>
<td>A few strains of Bacteroides, Ruminococcus torques, Bifidobacterium bifidum</td>
</tr>
</tbody>
</table>

* For structures and composition, see References 8 and 9. † References 10 to 12.
factors such as accessibility of polysaccharides in plant cell walls, competition for substrates, low metabolic rates or the presence of inhibitory substances could affect the amount of polysaccharide that is utilized by bacteria in vivo. To obtain an estimate of the extent to which polysaccharide utilization actually occurs in the colon, we compared the concentration of water soluble, high molecular weight carbohydrate in human ileal contents (i.e., in material about to enter the colon) with the concentration of high molecular weight carbohydrate in contents of various segments of the colon (14). Intestinal contents from four human subjects were analyzed and in all four cases the concentration of high molecular weight carbohydrate in the colon contents was several times lower than that in the ileal contents. These results indicate that appreciable breakdown of polysaccharides does occur in the human colon.

**Polysaccharide-degrading activities in human feces**

Further evidence that polysaccharides are being degraded in the colon comes from measurements of polysaccharide-degrading activity in human feces. A bacterial pellet was obtained by diluting fresh feces with phosphate buffered saline (0.05 M K-phosphate buffer, 0.9% NaCl, pH 7) and then centrifuging alternately at low speed (3,000 × g) and high speed (20,000 × g). The resulting bacterial pellet was resuspended in Na-citrate buffer (0.05 M, pH 6.8) to an optical density of 0.8 to 1.0 (at 650 nm) and the bacteria were disrupted by sonication. After centrifugation to remove intact bacteria, several types of polysaccharide-degrading activity were measured by following the increase in reducing sugar concentration when disrupted bacteria were incubated with the substrate at 37 C (15, 16). Polysaccharide-degrading activities found in three consecutive fecal specimens (A to C) and in a fourth specimen (D), taken after 5 days, are shown in Table 2. Xylanase and chondroitinase activities were higher than lichenase or polygalacturonase activities. This probably reflects the fact that xylan (from plant cell walls) and chondroitin sulfate (from dietary meat or sloughed epithelium) are available in the colon at higher concentrations than either lichenan (β-glucans) or polygalacturonic acid (pectins).

Several types of glycosidase activity were also measured (Table 3). Glycosidases are enzymes that cleave small molecular weight carbohydrates (e.g., disaccharides) into single sugars. These activities are frequently found in enzyme complexes responsible for polysaccharide breakdown. L-fucosidase activity is probably associated mainly with mucin breakdown, whereas the other enzyme activities are more characteristic of plant polysaccharide-degrading enzyme systems. The only glycosidase activity not detected in the fecal pellet was α-mannosidase. This is not too surprising in view of the fact that α-D-mannosides are much less common in nature than the other types of glycosides tested.

Both the polysaccharide-degrading activities and the glycosidase activities were relatively constant over the 10-day period covered by the study. No attempt was made to study the effect of a change in diet on these enzyme activities. However, Dr. George

**TABLE 2**

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Specific activity (μmol glucose per 100 min per mg protein)</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>A</td>
</tr>
<tr>
<td>Chondroitin sulfate</td>
<td>0.8</td>
</tr>
<tr>
<td>Lichenan</td>
<td>0.4</td>
</tr>
<tr>
<td>Polygalacturonate</td>
<td>0.3</td>
</tr>
<tr>
<td>Xylan</td>
<td>0.9</td>
</tr>
</tbody>
</table>

* Consecutive specimens; specimen D was taken 5 days after specimen C. All specimens were from the same individual.

**TABLE 3**

<table>
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</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A</td>
</tr>
<tr>
<td>α-D-mannoside</td>
<td>0</td>
</tr>
<tr>
<td>β-D-glucoside</td>
<td>1.3</td>
</tr>
<tr>
<td>β-D-galactoside</td>
<td>4.0</td>
</tr>
<tr>
<td>β-D-xyloside</td>
<td>0.7</td>
</tr>
<tr>
<td>α-L-fucoside</td>
<td>1.2</td>
</tr>
</tbody>
</table>

* Glycosidase activities were determined using nitrophenyl glycosides as substrates (17). Consecutive specimens; specimen D was taken 5 days after specimen C. All specimens were from the same individual.
Chang (Nutrition Department, University of California, Berkeley, Calif.) has measured glycosidase activities in the feces of people eating different types of diets. He found that people eating a high-fiber diet (bran) or a corn-bean diet had increased levels of α-galactosidase and β-glucosidase, compared to people eating a North American formula diet (private communication). In the feces of people who were receiving an egg-white supplement, he also detected an increase in L-fucosidase. L-fucose is a component of the ovo-mucin found in egg whites.

Enzyme assays may provide a useful, relatively simple method for monitoring the response of the intestinal flora to changes in diet. In order to interpret these results properly, however, we need to know more about the cellular location of the enzymes (i.e., cell-associated versus extracellular) and the conditions under which they are produced by typical strains of colon bacteria. If most of these enzymes are produced only in response to the presence of specific polysaccharide, enzyme assays performed on fecal preparations could be used to estimate the concentration of accessible polysaccharide in feces.

Mechanisms of polysaccharide breakdown in Bacteroides

Strategies used by bacteria for degrading polysaccharides appear to depend as much on the nature of the substrate as on the bacterial species. Bacteroides strains degrade linear, soluble polysaccharides such as chondroitin sulfate and laminarin with enzymes which are cell-associated rather than extracellular (15,18). In the case of chondroitin sulfate breakdown by B. ovatus, these enzymes appear to be periplasmic, since spheroplast formation by lysozyme-EDTA treatment releases all of the chondroitin sulfate degrading activity from the cells (A. Salyers, unpublished data). The laminarin-degrading enzymes of at least two Bacteroides species may also be periplasmic since sonically disrupted cells degraded laminarin more rapidly than do intact bacteria (15). Xylan, a branched, partially insoluble polysaccharide, also appears to be degraded by cell-associated enzymes (18), but the location of these enzymes has not been determined.

The main products of the laminarin-degrading enzymes from Bacteroides are small molecular weight compounds, e.g., glucose, laminaribiose, laminaritriorose (15). Similarly, the main product of the xylanases from Bacteroides appears to be the monosaccharide, xylose (18). It is probably advantageous for bacteria which produce enzymes that degrade polysaccharides into mono- or disaccharides, to have these enzymes closely associated with the cell so that the products of enzymic breakdown can be transported immediately. Many competing bacteria that cannot degrade laminarin or xylan can utilize glucose, xylose or disaccharides containing these sugars.

Extracellular enzymes are produced by Bacteroides in response to some polysaccharides such as guar gum (18) and psyllium hydrocolloid (19). Both of these compounds are highly viscous and extracellular enzymes may be necessary to reduce the viscosity sufficiently to permit attack by cell-associated enzymes. The extracellular guar gum-degrading enzyme produced by B. ovatus rapidly reduces the viscosity of guar gum. However, in contrast to the xylanases and laminarinases from Bacteroides, the guar gum-degrading enzyme does not yield small molecular weight products (monosaccharides, disaccharides). Rather, the products appear to have molecular weights of at least 100,000 daltons (J. Balascio, J. K. Palmer, and A. Salyers, unpublished data). These large pieces may be degraded further by cell-associated enzymes. They are probably not utilized readily by competing bacteria.

Control of enzyme production

Bacteroides that are grown in medium containing glucose or other monosaccharides as the carbon source have extremely low levels of polysaccharide-degrading activity. But when these bacteria are exposed to a fermentable polysaccharide, large increases in the level of polysaccharide-degrading activity are observed. An example of this is shown in Figure 1. Cells of B. ovatus grown on glucose or glucuronic acid have levels of chondroitinase activity which are close to the limit of detection. When these bacteria are inoculated into medium containing chondroitin sulfate, enzyme activity increases rapidly during early
logarithmic phase, then levels off during late logarithmic phase. Addition of glucose to the medium reduces the amount of chondroitinase produced, but an appreciable increase in specific enzyme activity is still detected. A similar pattern of induction by the polysaccharide and repression by glucose is also found with xylan-degrading enzymes from *B. ovatus* and other Bacteroides species.

Enzyme activities can be induced in cells of *B. ovatus* that are not actively growing. *B. ovatus* cells, grown on glucose and harvested during late logarithmic phase will produce chondroitinase or xylanase activity if they are resuspended (10<sup>10</sup> cells/ml) in medium containing the appropriate polysaccharide and incubated at 37°C. Chondroitinase activity appears within 30 min, xylanase activity within 2 hr. During this time, there is no significant increase in turbidity or total cell protein. Protein synthesis inhibitors, such as clindamycin or chloramphenicol, prevent the production of enzyme activity, so protein synthesis is required. The ability to produce degradative enzymes rapidly in response to a particular polysaccharide substrate is probably very useful in the colon since colon bacteria must obtain energy from a constantly changing mixture of polysaccharides.

**Conclusions**

To date, most studies of polysaccharide utilization by human colonic bacteria have focused on determining which species are polysaccharide degraders and what types of enzymes are involved in the breakdown of individual polysaccharides. The results of these studies have provided some useful information about the metabolic capabilities of colon bacteria. In the future, however, more emphasis needs to be placed on the process of polysaccharide utilization by intact bacteria, with special attention given to the effect on this process of conditions imposed by the colonic environment. Since the human colon resembles a continuous culture system more closely than a batch culture, we need to apply the continuous culture techniques which have been developed by rumen bacteriologists (20) to studies of polysaccharide catabolism by human colonic bacteria. Continuous cultures can mimic, to some extent, the special conditions imposed by the colonic environment, e.g., low growth rates and limiting substrate concentrations. Information obtained from continuous culture experiments should help us to design better experiments for probing the effect of diet on the bacterial flora of the colon.

**References**

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