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Digestion of Larch Arabinogalactan by a Strain of Human Colonic *Bacteroides* Growing in Continuous Culture

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Bacteroides thetaiotaomicron, a polysaccharide-degrading bacterium from the human colon, was grown in continuous culture, with arabinogalactan as the limiting substrate, at growth rates similar to those which are probably experienced by these organisms in vivo. Growth yields were respectively 80, 82, 58, and 50 g of cells/mol of utilized substate (as galactose) at growth rates of 3.5, 6.3, 11.6, and 27.7 h/generation. These yields were comparable to those attainable when glucose or galactose was the substrate. However, affinity for arabinogalactan was lower than affinity for galactose. As the rate of growth decreased, the pattern of fermentation products changed: the concentration of acetate and propionate increased and the concentration of succinate decreased. The ability of the bacteria to produce the inducible enzyme α-glucosidase also decreased with decreasing growth rate, indicating that slowly growing bacteria may be less able to adapt to new sources of carbohydrate.

Many of the bacteria which reside in the human colon require a fermentable carbohydrate for growth (Holdeman et al., 1977). A number of these organisms can ferment polysaccharides (Salyers et al., 1977a,b), and polysaccharides from the host's diet or from the host's secretions may be the only fermentable carbohydrates available to these organisms in their natural environment. There is considerable evidence to indicate that extensive bacterial digestion of some types of dietary polysaccharides occurs in the colon (Van Soest, 1978). However, little is known about the factors which affect the extent of this digestion, the types of polysaccharides most likely to be attacked, and the effect of this microbial digestion on the host. Some understanding of these factors is necessary in order to answer such questions as (a) whether colon bacteria live mainly off their host or off their host's diet, (b) whether the composition of the host's diet (especially its fiber content) affects the extent to which bacteria utilize host-produced substances as a source of carbohydrate, (c) in what ways bacterial digestion affects the properties of dietary fiber and thus the action of fiber in the colon, and (d) whether products from bacterial fermentation of substances which are indigestible by the host are absorbed and whether this absorption contributes substantially to the host's nutrition.

Since the bacterial flora of the colon is an extremely complex community of interdependent organisms (Moore and Holdeman, 1974; Wolin, 1974), there are undoubtedly a large number of factors which influence its composition and metabolic activities. The nature of available carbohydate is likely to be a major factor affecting the functioning of this community because so many bacteria rely either directly on carbohydates or on the products of carbohydate fermentation by other organisms. At any one time, the concentration of any particular type of polysaccharide in the colon is probably quite low. Moreover, the polysaccharide pool, which is composed of a complex mixture of different types of polysaccharides, is constantly changing. Because concentrations of substrates are low, growth rates are probably low as well (Brock, 1971). The transit time of most materials through the colon is around 30–40 h. Thus, the bacterial mass, which is stable and accounts for nearly one-third of the volume of colon contents, can replace itself only once during this time. Bacteria which are located in the ascending colon near the ileocecal valve where dietary material enters the colon probably experience much higher growth rates than bacteria which are further along in the colon. However, even these growth rates are likely to be much slower than the growth rates of 30 min–2 h/generation which are customary in most in vitro systems used by microbiologists to study the metabolic activities of bacteria. To date, no direct measurements have been made of growth rates of human colonic bacteria, but measurements have been made of bacterial growth rates in the cecum and colon of rodents. Growth rates of the organisms tested were found to be on the order of 10 h/generation or longer (Gibbons and Kapsimalis, 1967).

Virtually nothing is known about the effect of very slow growth rates on the ability of colon bacteria to utilize complex substrates such as polysaccharides or on their ability to produce new degradative enzymes in order to shift from one substrate to another as the composition of available carbohydrate changes. To obtain some insight into the effect of slow growth rates on bacterial digestion of polysaccharides, we have investigated the utilization of larch wood arabinogalactan by a strain of *Bacteroides thetaiotaomicron* growing in continuous culture with arabinogalactan as the limiting nutrient. Larch wood arabinogalactan is a mixture of two component polysaccharides, one having a molecular weight of around 160 000 and the other having a molecular weight of around

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100 000 (Whistler and Richards, 1972). Both components are characterized by a $\beta(1,3)$-linked galactan backbone with side chains containing galactose and arabinose. Larch wood arabinogalactan was chosen for this study because it is soluble in water, is commercially available, and has a complex, branched structure comparable to that of many other plant cell wall polysaccharides. *B. thetaiotaomicron*, one of the major species in the colonic microflora (Moore and Holdeman, 1974), is able to ferment a variety of polysaccharides, including arabinogalactan (Salyers et al., 1977a).

Continuous cultures were used in this study in order to approximate some of the conditions encountered by colon bacteria in their normal habitat. In continuous culture, fresh medium is pumped into the culture vessel at a given rate and bacteria and spent medium are removed at the same rate. When some nutrient, in this case the arabinogalactan, is growth limiting, the rate at which medium is supplied determines the growth rate of the organisms in the vessel. Moreover, the same growth rate can be maintained over many generations. To determine the effect of very slow growth rates, we equilibrated the bacteria at steady-state growth rates of 3.5, 6.3, 11.6, or 27.7 h/generation. At each growth rate, the bacterial yield was determined and used as an indication of how efficiently the bacteria were using the arabinogalactan. The ability of the bacteria to produce enzymes for degrading a new carbohydrate source was determined by measuring the rate at which α-glucosidase, an inducible enzyme, was produced when bacteria were exposed to maltose. Finally, the spent medium was analyzed to determine whether any undegraded portions of the arabinogalactan remained and, if so, what were the properties of this residual carbohydrate.

Although growth rates in the colon are probably relatively slow, we were also interested in determining how efficiently *B. thetaiotaomicron* could utilize a substrate as complex as arabinogalactan at more rapid growth rates. Accordingly, we measured the amount of arabinogalactan which was utilized at growth rates ranging from 1.3 to 3.5 h/generation and compared the results with those obtained at similar growth rates when galactose was the substrate.

MATERIALS AND METHODS

Bacterial Strain and Growth Conditions. *B. thetaiotaomicron* VPI 5482A (NCTC 10852) was obtained from the culture collection of the Anaerobe Laboratory, Virginia Polytechnic Institute and State University, Blacksburg, VA. The defined medium used in these experiments was based on the defined medium described by Varel and Bryant (1974). The medium consisted of arabinogalactan (0.2% w/v), potassium phosphate buffer (0.05 M, pH 7.0), $(NH_4)_2SO_4$ (10 mg/mL), NaCl (9 mg/mL), $CaCl_2$ (0.26 mg/mL), $MgCl_2$ (0.20 mg/mL), $MnCl_2$ (0.10 mg/mL), $CoCl_2$ (0.10 mg/mL), $FeSO_4$ (4 μg/mL), hemin (10 μg/mL), vitamin B_{12} (5 ng/mL), cysteine (0.5 mg/mL), and Na_2CO_3 (0.4% w/v). Larch wood arabinogalactan (Sigma Co., St. Louis, MO) and cysteine hydrochloride were filter sterilized and added to the medium after autoclaving. Autoclaved sodium carbonate was also added separately to a final concentration of 0.4%. The continuous culture system used in these experiments was similar to that described by Kafkewitz et al. (1973), except that the temperature in the culture vessels were maintained at 37 °C by heating tape wrapped around the vessels rather than by a circulating water pump. The volume of the culture vessel was 95 mL. The atmosphere in the vessels and in the reservoir was oxygen-free carbon dioxide. At each dilution rate, a separate culture vessel was inoculated with a stock culture in chopped meat broth (Holdeman et al., 1977). All stock cultures were derived from a single colony of 5482A. After inoculation and growth, bacteria were equilibrated at the dilution rate under investigation for five to seven generations before collection and analysis. The order of dilution rates was 0.20, 0.025, 0.06, 0.11, and 0.20 h^{-1}. The 0.20-h^{-1} dilution rate was repeated with a separate culture to make sure that the medium in the reservoir had not deteriorated.

The concentration of larch arabinogalactan in medium supplied to the cells was 1.7 mg/mL as determined by phenol–sulfuric assay (Dubois et al., 1956), with galactose as a standard. This concentration of arabinogalactan supported growth of *B. thetaiotaomicron* to a concentration which was about half that attainable in batch culture when arabinogalactan was in excess. The concentration of bacteria was proportional to the concentration of arabinogalactan in the range of the concentrations used in these experiments. Thus arabinogalactan was the limiting nutrient in the medium supplied to the cells.

Chemical Analyses of Bacteria and Fermentation Products. Dry weights of bacteria were obtained by vacuum filtration of cells (15 mL) onto Millipore DAWP filters (0.65-μm pore size; 47-mm diameter). Bacteria on the filter were washed with 10 mL of potassium phosphate buffer (0.05 M, pH 7). Before use, filters were heated at 110 °C for 3 h, placed in a desiccator under vacuum for 30 min, and then weighed. Bacteria trapped on these tared filters were dried and weighed in the same manner. For determinations of DNA, RNA, and protein concentrations, bacteria (5 mL) were removed from the culture vessel into ice-cold 3 M trichloroacetic acid (1 mL). After 1.5 h on ice, the precipitate was collected by centrifugation and then washed once in 0.2 M trichloroacetic acid. The DNA concentration in the air-dried precipitate was determined by the diphenylamine assay (Burton, 1955), with deoxyribose as the standard. For RNA and protein determinations, the air-dried precipitate was solubilized in 0.2 N NaOH. RNA concentrations were determined by the orcinol assay (Schneider, 1967) with ribose as the standard. The multiplicative factors used for converting ribose concentrations to RNA concentrations and deoxyribose concentrations to DNA concentrations were 4.9 and 2.44, respectively. Protein concentrations were determined by using the method of Lowry et al. (1951), with bovine serum albumin as the standard. Total cell carbohydrate was estimated by phenol–sulfuric assay (Dubois et al., 1956) of bacteria which had been harvested by centrifugation (15000*g*, 15 min, 4 °C), washed once with 0.05 M potassium phosphate buffer (pH 7.0), and then disrupted by sonication (2 min). Concentrations of carbohydrate left undigested by the bacteria were determined by phenol–sulfuric assay of culture fluid from which cells had been removed by centrifugation. Concentrations of fermentation products were determined by gas–liquid chromatography of the butylated derivatives (Salanitro and Muirhead, 1975).

Chemical Analysis of Arabinogalactan. Medium, either uninoculated or spent, was first passed over a 1 × 3 cm column of Amberlite MB-3 mixed bed resin and then neutralized with NaOH. Since the concentrations of residual carbohydrate in spent medium were relatively low, spent medium was concentrated 10-fold by flash evaporation (30 °C) after treatment with Amberlite. Portions (1 mL) of this concentrated, desalted sample were chromatographed on a 1.5 × 85 cm column of Bio-Gel A 0.5m, with distilled water as the eluant. The flow rate was 20 cm/h and fractions of 1.5 mL were collected. The concentration of carbohydrate in each fraction was determined

Table I. Effect of Dilution Rate on Dry Weight and Composition of Bacteria

dilution rate, h^{-1}:[a] generation time, h:[b]	0.20 3.5	0.11 6.3	0.06 11.6	0.025 27.7
dry wt of bacteria, mg/mL of culture	0.50 (±0.02)[c]	0.54 (±0.03)	0.42 (±0.01)	0.38 (±0.02)
growth yield, mg dry weight/mmol of substrate, as galactose	80	82	58	50
protein, mg/mL	0.35 (±0.03)	0.36 (±0.03)	0.33 (±0.02)	0.31 (±0.03)
carbohydrate, μg/mL	50 (±2)	41 (±3)	33 (±3)	35 (±2)
DNA, μg/mL	11.3 (±0.4)	11.3 (±0.2)	10.6 (±0.9)	8.9 (±0.8)
RNA, μg/mL	48 (±4)	44 (±4)	39 (±2)	26 (±1)
RNA/DNA	4.2	3.9	3.7	2.9

[a] Dilution rate = [flow rate of medium into culture vessel (mL/h)]/[volume of culture vessel (mL)]. [b] Generation time = 0.693/dilution rate. [c] Values in parentheses are average deviations from the mean. Mean values were averages of duplicate determinations from at least two separate culture vessels.

by phenol–sulfuric assay. When arabinogalactan in uninoculated medium was taken through the desalting and concentration procedures outlined above, its chromatographic profile was identical with that of arabinogalactan placed directly on the column without pretreatment. Thus the desalting and evaporation steps did not appear to alter the arabinogalactan.

For further analysis, fractions from the Bio-Gel A 0.5m column were pooled, concentrated by flash evaporation, and then hydrolyzed in 2 N trifluoroacetic acid (TFA) for 2 h at 100 °C under nitrogen. After hydrolysis, the sample was evaporated to dryness and then washed several times in methanol to remove the TFA. The pH of the sample, when it was resuspended in distilled water, was around 5. Monosaccharides in this hydrolyzed sample were resolved by chromatography on Whatman 1 paper with butanol–acetone–water (4:5:1 v/v/v) as the solvent system. Sugars were visualized by spraying the chromatograms with *p*-anisidine (0.1 M) in pthalic (ethanolic) acid (0.1 M) and then heating at 100 °C for 10 min. Relative amounts of galactose and arabinose in each fraction were estimated by chromatography of the [^{3}H]borohydride-reduced sugar alcohols on DEAE paper (Conrad et al., 1973).

Induction Studies. To determine the effect of growth rate on the ability of *B. thetaiotaomicron* to respond to a new substrate, we measured the induction of α-glucosidase synthesis by maltose. Since previous studies had shown that protein synthesis in *B. thetaiotaomicron* is sensitive to oxygen and to centrifugation and resuspension of cells, even under anaerobic conditions (Salyers and Kotarski, 1980), bacteria (9.5 mL) were removed anaerobically from the culture vessel directly into tubes which had been gassed out with oxygen-free carbon dioxide and which contained 0.5 mL of maltose (100 mg/mL). The mixture was incubated at 37 °C. At intervals, portions (2 mL) of the incubation mixture were removed into 3 mL of ice-cold potassium phosphate buffer (0.05 M, pH 7.0) to stop the induction process. After centrifugation, the supernatant fluid was discarded. Bacteria were then resuspended in phosphate buffer (0.05 M, pH 7.0) and disrupted by pulsed sonication (50%) for 2 min. During sonication, bacteria were kept cold in an ice–water bath. After sonication, cell debris was removed by centrifugation and the supernatant fluid was tested for α-glucosidase activity. α-Glucosidase activity was determined by measuring the increase in absorbance at 370 nm when enzyme was incubated at 37 °C with *p*-nitrophenyl-α-D-glucoside (4 mM in 0.05 M phosphate buffer, pH 7). Enzyme activity was linear throughout the assay period and was proportional to the amount of enzyme used in the incubation. Protein concentrations were determined by using the method of Lowry et al. (1951). Duplicate induction experiments were carried out with cells from each culture vessel. At least two different culture vessels were analyzed at each dilution time. The rate of induction was not affected by addition of enough succinate, acetate, or propionate to increase the concentration of volatile fatty acids by 10 mM. Nor was the rate of induction affected by addition of arabinogalactan to a final concentration of 1 mg/mL. Thus, rates of induction at different dilution rates could be compared despite differences in concentrations of volatile fatty acids or residual carbohydrate in the extracellular fluid.

Affinity Studies. To determine how efficiently *B. thetaiotaomicron* could utilize arabinogalactan at rapid growth rates, we equilibrated the bacteria for at least 5 generation times at growth rates ranging from 1.3 to 7.0 h/generation (dilution rates from 0.5 to 0.1 h^{-1}). The concentration of arabinogalactan in the medium was 1.9 mg/mL. For comparison, bacteria were grown at similar rates in medium containing galactose (2.0 mg/mL) as the sole carbohydrate source. The concentration of unused substrate in the extracellular fluid was determined by phenol–sulfuric assay. The affinity constant (K_s) was calculated from a double-reciprocal plot of $1/S$ vs. $1/D$, where S was the concentration of unused carbohydrate and D was the dilution rate (Russell and Baldwin, 1979).

RESULTS

The concentration of viable bacteria in the culture vessel [(1.7–1.9) × 10^9/mL] was the same at all dilution rates. Viability was approximately 100%, since the total concentration of bacteria, as determined by using a Petroff-Hauser chamber, was 1.8 × 10^9/mL. Although the concentration of bacteria did not change significantly with dilution rate, the dry weight of the bacteria (or, equivalently, the growth yield) decreased as dilution rate decreased (below 0.11 h^{-1}) (Table I). The growth yields in Table I were computed by expressing the amount of arabinogalactan utilized as moles of equivalent galactose. This is reasonable since arabinose accounts for less than 10% of the total sugar in arabinogalactan. Expressing the yields in this way permits them to be compared with yields of bacteria growing on monosaccharides. The molar growth yields shown in Table I are comparable to the growth yields obtained when *B. thetaiotaomicron* 5482A was grown under similar conditions with glucose or galactose as the limiting substrate (A. Salyers, unpublished data).

Total cellular carbohydrate declined as growth rates became slower (Table I). This may reflect a decrease in storage polysaccharide. The concentration of RNA and the ratio of RNA to DNA also declined with decreasing dilution rates. Since most of the bacterial RNA is ribosomal RNA, the decrease in RNA concentration probably indicates that at slower growth rates the number of ribosomes per cell decreases. This possibility is also supported by the results of the induction experiments (Figure 1). The rate at which the synthesis of α-glucosidase is induced

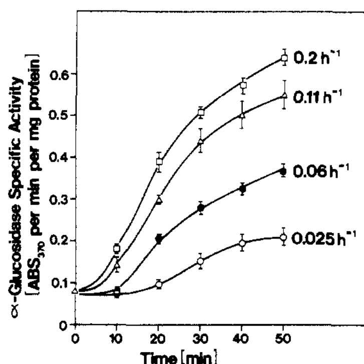

Figure 1. Induction of α-glucosidase in bacteria growing at dilution rates of 0.20, 0.11, 0.06, or 0.025 h^{-1}. Maltose, the inducer, was added at 0 min to bacteria taken from the continuous cultures. The initial concentration of maltose was 5 mg/mL. The initial concentration of bacteria was (1.7–1.9) × 10^9/mL at all dilution rates.

Table II. Effect of Dilution Rate on Concentrations of Major Fermentation Products

fermentation product	concn, mM, at a dilution rate, h^{-1}, of			
	0.20	0.11	0.06	0.025
acetate	2.1 (±0.2)[a]	4.0 (±0.3)	6.2 (±0.4)	5.9 (±0.4)
succinate	2.8 (±0.2)	3.2 (±0.2)	2.5 (±0.5)	2.1 (±0.2)
propionate	0.4 (±0.1)	1.3 (±0.2)	3.0 (±0.3)	3.7 (±0.2)

[a] Values in parentheses are average deviations from the mean. Mean values were averages of duplicate determinations from at least two separate culture vessels.

when bacteria were exposed to maltose was much lower when bacteria were growing with a generation time of 27.7 h than when they were growing with a generation time of 3.5 or 6.3 h. The decline in dry weight between the dilution rate of 0.11 h^{-1} and the dilution rate of 0.025 h^{-1} was not completely accounted for by the decline in protein, carbohydrate, and nucleic acids. This may indicate that concentrations of other important cell components (e.g., lipids) also decline significantly.

The relative amounts of the three major fermentation products shifted as the growth rate decreased (Table II). At a generation time of 3.5 h (dilution rate of 0.2 h^{-1}), the main fermentation products were acetate and succinate. Concentrations of acetate and succinate were nearly equal. As the growth rate decreased to 27.7 h (dilution rate of 0.025 h^{-1}), the concentration of propionate increased relative to that of succinate and acetate, and the concentration of succinate decreased relative to that of acetate so that the ratio of acetate to succinate became nearly 3:1.

Although the concentration of arabinogalactan was growth limiting, there was carbohydrate detectable in the medium at all dilution rates (Table III). The amount of this carbohydrate decreased from 34% of the total available carbohydrate at the highest growth rate (dilution rate = 0.20 h^{-1}) to 18% of the total available carbohydrate at the lowest growth rate (dilution rate = 0.025 h^{-1}). The chromatographic profile of this residual carbohydrate is compared to the profile of undegraded arabinogalactan in Figure 2. Undegraded arabinogalactan was resolved by chromatography on Bio-Gel A 0.5m into two major peaks (Figure 2, panel A), one of which eluted in the void volume (peak I) and one of which eluted somewhat later (peak II). The ratio of peak I to peak II was approximately 1:2 (Table III). TFA hydrolysis of both of these peaks, followed by resolution of the component sugars on Whatman I paper, revealed that the main component of each of these peaks was galactose. A small amount of arabinose (7–9% of total carbohydrate) was also present in each peak. No other

Table III. Effect of Dilution Rate on the Amount and Molecular Weight Distribution of Arabinogalactan Not Digested by Bacteria

	uninoculated medium	spent medium from organisms growing at a dilution rate, h^{-1}, of			
		0.20	0.11	0.06	0.025
carbohydrate[a] in medium, mg/mL	1.7	0.57	0.51	0.39	0.33
% in					
peak I	34[b]	31	32	32	38
peak II	64	34	38	34	33
peak III	2	27	21	24	23

[a] Total carbohydrate determined by phenol–sulfuric assay (Dubois et al., 1956). [b] For definition of peaks I, II, and III, see Figure 2 and the text. Percentages were estimated by adding up the micrograms of carbohydrate in fractions comprising a particular peak, dividing this value by the total micrograms of carbohydrate recovered from the column, and multiplying by 100. At least 85% of the phenol–sulfuric carbohydrate detected in the medium was recovered in peaks I, II, and III.

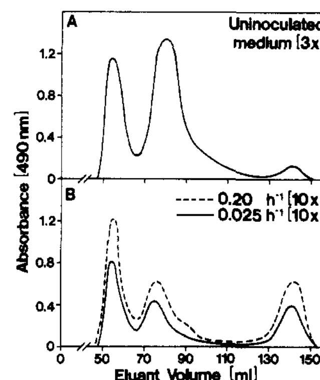

Figure 2. The chromatographic profile on Bio-Gel A 0.5m (1.5 × 85 cm) of larch arabinogalatan in uninoculated medium displayed two major peaks (panel A). Peak I includes eluant volume 50–65 mL. Peak II includes eluant volume 65–95 mL. The void volume of the column was 55 mL and the fully included volume was 145 mL. The chromatographic profile of residual carbohydrate after digestion of arabinogalactan (panel B) also displayed peaks in the same elution volumes as I and II. A third peak (III) was detected in the fully included volume of the column (130–150 mL). The x-fold concentration of medium or extracellular prior to chromatography is given in brackets. At least 85% of the phenol–sulfuric carbohydrate detected in the medium was recovered in peaks I, II, and III.

sugars were seen, even when the paper chromatogram was overloaded. Residual carbohydrate from spent medium was resolved into three peaks (Figure 2, panel B). One peak eluted in the same volume as peak I of the undegraded arabinogalactan. The second peak eluted in the same volume as peak II. However, the ratio of peak I to peak II in the residual carbohydrate was 1:1; i.e., peak I accounted for a higher proportion of the residual carbohydrate than of the uninoculated arabinogalactan (Table III). The relative proportions of peak I and II did not vary significantly with dilution rate. Hydrolysis of peaks I and II from the residual carbohydrate revealed that, as with peak I and II from arabinogalactan, galactose was the major component and arabinose was the only other sugar detected in the hydrolysate. Arabinose accounted for 8% of peak I and 7% of peak II. Thus the arabinose content of residual arabinogalactan was essentially the same as that of undegraded arabinogalactan.

The third peak in the residual carbohydrate (peak III) eluted in the fully included volume. A small amount of

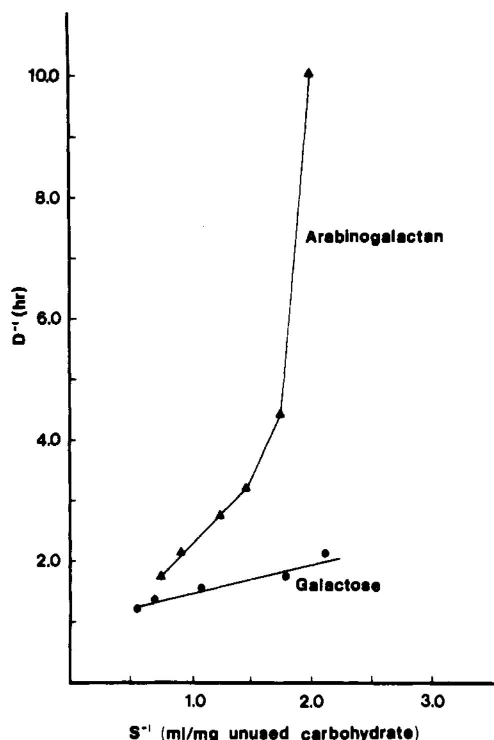

Figure 3. A double-reciprocal plot for *B. thetaiotaomicron* growing in medium containing either arabinogalactan (1.9 mg/mL) or galactose (2.0 mg/mL) as the limiting carbohydrate source. S is the concentration of unused carbohydrate remaining in the medium and D is the dilution rate.

this substance was also detectable in uninoculated medium. When peak III was rechromatographed on Bio-Gel P2 (1.5 × 65 cm) with water as eluant, it eluted as a single peak within the column and eluted prior to maltotriose (data not shown). Thus it is probably larger than a trisaccharide but not large enough to be excluded totally by the Bio-Gel P2 resin. The TFA hydrolysate of this fraction contained both galactose and arabinose. Arabinose accounted for 9% of the total carbohydrate.

The fact that the yield at 3.5 h/generation was somewhat lower than the yield at 7.0 h/generation (Table I) indicated that we might be getting close to the maximum growth rate possible for *B. thetaiotaomicron* when arabinogalactan was the substrate. To determine how fast these bacteria could grow on arabinogalactan and to compare their affinity for arabinogalactan with their affinity for galactose, we determined the optical density (650 nm) and concentration of unused substrate at growth rates ranging from 1.3 to 7.0 h/generation (arabinogalactan) or from 1.1 to 5.0 h/generation (galactose). At a growth rate of 1.3 h/generation, the optical density had dropped to 37% (arabinogalactan) and 75% (galactose) of that at 3.5 h/generation. A double-reciprocal plot of $1/D$ vs. $1/S$, where D is dilution rate and S is concentration of unused substrate, is shown in Figure 3 for bacteria growing on arabinogalactan and on galactose. In Figure 3 only values for growth on galactose at rates ranging from 1.1 to 1.5 h/generation are shown because the values of $1/S$ became too large to plot conveniently on the same scale as the arabinogalactan values. The $1/D$ vs. $1/S$ plot was still linear for growth on galactose at the slower rates which are not shown in Figure 3. At rapid growth rates, the relationship between dilution rate and substrate concentration for most bacteria follows Michaelis kinetics (Russell and Baldwin, 1979). In the case of *B. thetaiotaomicron* this was true for galactose and for growth rates up to about 2.2 h/generation when arabinogalactan was the substrate. At slower growth rates, the arabinogalactan curve departed from linearity. An affinity constant, K_s, calculated from the linear portion of the arabinogalactan curve was 7.03 mg/mL, whereas the K_s for galactose was 0.4 mg/mL. Thus the affinity of *B.* thetaiotaomicron for galactose was much higher than affinity for arabinogalactan.

DISCUSSION

A single organism utilizing a single type of polysaccharide in continuous culture is too simple a system to serve as an accurate model for the process of polysaccharide digestion by the microflora of the human colon. However, the results of experiments done with such a system can be used to derive general principles of bacterial function which may help us to understand what actually occurs in the colon.

To our knowledge, this study represents the first attempt to investigate polysaccharide breakdown by anaerobic bacteria growing in continuous culture. Several conclusions can be drawn from the results of this study. First, growth yields on arabinogalactan were quite high. In fact, they were comparable to yields obtainable when a monosaccharide such as glucose or galactose was the substrate (A. Salyers, unpublished results). Thus, *B. thetaiotaomicron* can utilize efficiently a complex substrate such as arabinogalactan over a wide range of growth rates. Although yields of bacteria growing on the polysaccharide were comparable to yields of bacteria growing on the monosaccharide, the affinity of *B. thetaiotaomicron* for arabinogalactan was much lower than its affinity for a monosaccharide such as galactose, as indicated by the fact that the concentration of unutilized substrate increased much more rapidly with increasing growth rates when arabinogalactan was the substrate than when galactose was the substrate. Moreover, the slope of the double-reciprocal plot of $1/D$ vs. $1/S$ for arabinogalactan was not constant, as was the case with galactose, but increased at growth rates slower than 2.2 h/generation. This could have been due to the existence of a fraction of arabinogalactan molecules which were less digestible, e.g., because they contained more branches per molecule. Alternatively, the increase in slope could have been due to high maintenance energy expenditures (Russell and Baldwin, 1979). Further work to determine affinities of *B. thetaiotaomicron* for other polysaccharides is currently underway in our laboratory. The results of these experiments may help us to predict what sorts of polysaccharides are likely to be utilized preferentially, since affinity is likely to be a factor in determining probability of utilization.

Second, the relative amounts of the products of bacterial fermentation can vary with growth rate. Differences in the pattern of fermentation products may be important to the host if some products, such as acetate, are absorbed more readily than others. Moreover, the balance between the polysaccharide degraders and other species of bacteria which utilize their products (e.g., succinate or acetate utilizers) could be affected by the amount of these substances which is produced at a given growth rate.

A third conclusion which can be drawn from the results of this study is that microbial digestion of a polysaccharide does not necesarily mean that the polysaccharide merely disappears from the medium. Relatively indigestible portions of the polysaccharide, of large or small molecular weight, can be left behind. This mixture can be quite complex. When *Bacteroides* species degrade arabinoxylans, for example, a variety of fragments, from disaccharides to much higher oligomers, is left behind in the medium (F. Gherardini and A. Salyers, unpublished results). Thus when dietary fiber components are degraded

by colonic bacteria, the original set of polysaccharides may be replaced by a different mixture of polysaccharides and oligomers, and the properties of this new mixture (e.g., the ability to bind ions or toxic substances) may be very different from the properties of the original mixture. This possibility should be taken into account by workers who are investigating properties of dietary fiber components which may have physiological importance in the human intestinal tract.

Finally, the ability of the organisms to produce the enzymes necessary to enable them to switch to a new carbohydrate source was significantly decreased when generation times were long. If this is true in general for colon bacteria, bacteria which are capable of utilizing either dietary polysaccharides or polysaccharides in host secretions may be more likely to utilize polysaccharides which are always available, i.e., the polysaccharides in host secretions, rather than polysaccharides from the diet which are much more variable with respect to availability and digestibility. The effect of diet, and especially of the digestibility of dietary components by colonic bacteria, on the extent to which these bacteria degrade host mucins and other host secretions needs to be investigated since bacterial degradation of these substances might have some impact on the health of the host.

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Enzymatic Degradation of the Plant Cell Wall by a *Bacteroides* of Human Fecal Origin

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The isolated cell wall of peanuts contained about 20% pectic substances, 20% cellulose, and 30% hemicelluloses. An inoculum of human feces was able to ferment the cell wall, and eight organisms capable of fermenting this substrate were subsequently isolated. One of these, identified as a *Bacteroides*, was selected for a study of the enzymatic mechanism of cell wall degradation. The cell wall degrading activity was predominantly intracellular and present at low levels in bacteria grown on glucose or xylose. The intracellular enzyme activity increased ~6-fold in bacteria grown on the peanut cell wall, and the increased activity was present within 4 h of inoculation into the cell wall medium. The intracellular enzyme fraction from bacteria grown on the cell wall liberated ~11% of the cell wall sugars during an 18-h incubation. Uronic acid and xylose were the predominant products. These results with a prototype dietary fiber indicate the potential for fiber degradation in the human colon, which could alter the properties of the fiber.

The physiological effects of dietary fiber are difficult to evaluate because of the complexity and variability of fiber composition. Many of the therapeutic properties which are attributed to dietary fiber pertain to its function in the colon. It has been postulated that hydrated fiber may cause a decrease in transit time of materials through the colon, may dilute potentially harmful compounds, or may actually bind certain classes of compounds, most notably bile salts and acids (Mitchell and Eastwood, 1976). Current research is attempting to relate the chemical structure and the physical properties of dietary fiber to its physiological function. There is evidence, however, which suggests that the chemical structure of dietary fiber may be modified during transit through the colon. The saccharolytic microorganisms which populate the colon may attack some polysaccharide components of dietary fiber. The evidence which supports such an alteration of dietary fiber

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