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Characterization of Plant Polysaccharide- and Mucin-Fermenting Anaerobic Bacteria from Human Feces

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Organisms able to grow on arabinogalactan, pectin, xylan, wheat bran, guar, apple cell walls, and mucin were isolated by enrichment from human feces. The number of polysaccharide fermenters and the properties of the predominant bacteria varied between subjects. The ability to use one polysaccharide was not related to the ability to use others. Some organisms (e.g., *Bacteroides* spp.) isolated on other substrates also utilized mucin, but were not isolated in the mucin enrichment. The mucin fermenters isolated by enrichment had a very restricted ability to utilize complex polysaccharides and their constituent monosaccharides, suggesting that the presence of plant polysaccharides in the human colon is unlikely to prevent the use of colonic mucin as an energy source by bacteria. Characterization with a range of biochemical tests showed that many of the isolates, but especially the mucin fermenters, did not resemble organisms described previously.

In humans the ingestion of plant cell wall polysaccharides (which have been described as dietary fiber) alters mouth-anus transit time, lumen pH, short-chain fatty acid production, fecal weight, and mucosal morphology (7).

In the colon some plant cell wall polysaccharides that have resisted enzymic digestion in the higher gut are fermented by the anaerobic bacterial flora, leading to increased production of short-chain fatty acids and so to increased energy available to the colonic epithelium and to multiplication of the bacteria that are excreted in the feces (25).

A large number of organisms isolated from feces have been tested in vitro for their ability to utilize polysaccharides (21, 22). Since polysaccharides such as pectin and arabinogalactan, together with host secretions, are the major sources of carbon and energy for colon bacteria, it is not surprising that, although there was great diversity among the species tested, many of the organisms were able to grow on these substrates or their constituent monosaccharides. However, of the 342 strains tested, only 6 (of 9) strains of *Ruminococcus torques* and 2 (of 5) strains of *Bifidobacterium bifidum* were able to ferment porcine gastric mucin, which resembles human colonic mucin in general composition (14), although fermentation of the monosaccharides present in mucin was widespread. Estimates of the utilization of porcine gastric mucin by human fecal incubates indicated that the population density of mucin-fermenting organisms in normal human subjects was about 1% of the total population of anaerobes (18); it has been suggested that host-produced polysaccharides may be a preferred carbon source for bacteria that can utilize them (16).

Few studies of the properties of the anaerobic colon flora have been carried out with methods that preferentially select for organisms in the population that can ferment polysaccharide substrates, although Dekker and Palmer (9) have shown that an organism isolated by an enrichment technique with isolated peanut cell walls as the carbohydrate source differed from previously described species. In the present study isolations with both selective and enrichment techniques from feces were used to determine the concentration and types of organisms that are capable of growth on polysaccharides or mucin and to observe whether any properties of these anaerobic bacteria might be related to their survival in the colon. Wheat bran and apple cell wall material (ACW) (characterized in Table 1) were chosen to represent fiber commonly ingested in the human diet, but which might vary in availability for bacterial fermentation, particularly since ACW contains a high proportion of uronic acid residues. Larchwood xylan and guar gum were chosen as plant cell wall preparations that would require relatively few enzymes for fermentation. Guar gum is utilized in the food processing industry and is therefore part of the average Western diet.

A range of biochemical tests was carried out on all of the isolates to highlight both the variation within each population and among selective and enrichment techniques, rather than to identify definitively.

MATERIALS AND METHODS

Substrates for growth. The alcohol-insoluble residues of wheat bran used by Cummings et al. (8) in clinical feeding trials were further milled in a laboratory grain mill (0.5-mm sieve). ACW was prepared from fresh apple cortex by extraction with 1.5% (wt/vol) sodium dodecyl sulfate containing 5 mM sodium metabisulphite (pH 5) in a ball mill for 6 h followed by extraction with 90% dimethyl sulfoxide for 17 h, after which the residue was extensively dialyzed and then freeze-dried. The guar gum preparation was a sample of Meyprogat 150 (a gift from Meyhall Chemical AG, Switzerland). Pectin (from citrus fruit), xylan (from larchwood), mucin (from porcine stomach), arabinogalactan, and monosaccharides were all from Sigma Chemical Co. The aldoses and uronic acid content of some of the cell wall preparations used in this experiment were obtained by the methods of Selvendran et al. (23) and are shown in Table 1.

Preparation of samples and enumeration of anaerobes. Fecal samples for experiments SC, TP, CB, G1, and G2 were provided by different adult volunteers, except for CB and G2, which were carried out on two different samples from the same volunteer. None had taken antibiotics within the previous 3-month period. Freshly voided feces were collected in plastic bags and immediately placed in an anaerobic cabinet (Forma Scientific) containing N_2-H_2-CO_2 (85:10:5, vol/vol). The feces were kneaded by hand, and a sample (0.5 to 1.5 g) was transferred to a weighed bottle containing 10 ml of anaerobic dilution solution (4); the bottle was reweighed, and the contents were mixed. Further dilutions for experiment SC were to 10^{-10} in anaerobic dilution solution for the

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TABLE 1. Aldoses and uronic acid content of substrates used for enrichment isolation

Substrate	Content (μg/mg [dry wt])						
	Deoxyhexose	Arabinose	Xylose	Mannose	Galactose	Glucose	Uronic acid
Bran[a,b]	22	180	200	Tr	11	141	Tr
ACW[c]	20	123	33	43	57	227	328
Xylan[a]	11	9	577	14	6	12	120
Guar[a]	1	8	1	436	229	9.4	

[a] After hydrolysis with 1 M H_2SO_4 for 2.5 h at 100°C (23).
[b] Data first published by Selvendran et al. (24).
[c] After Saemen hydrolysis (23).

enumeration of total anaerobes after 5 days of incubation on plates containing the complex VLhlf medium with glucose as the major carbon source together with hemin, liver extract, and fecal extract (5) or on the basal defined medium of Varel et al. (27), modified by the omission of sugars and volatile fatty acids, the replacement of Na_2S with 0.05% (wt/vol) cysteine hydrochloride, and the addition of 1% (wt/vol) NH_4Cl (BDM). The substrates under test (pectin, arabinogalactan, xylan, and glucose for experiment SC, 0.5%, wt/vol) were sterilized by autoclaving in the medium, apart from glucose, which was filter sterilized and added aseptically after autoclaving. All plates were prereduced, inoculated, and incubated in the anaerobic cabinet at 37°C. Strict anaerobiosis was maintained at all other times by using the techniques of Hungate (12, 13). Counts of anaerobes on BDM-glucose gave a recovery similar to that on VLhlf.

For enrichment, samples were diluted in BDM prepared under CO_2 gas phase with the omission of agar and the addition of substrates as indicated. For experiment TP isolations from the 10^{-8}, 10^{-9}, and 10^{-10} dilutions of BDM-bran, BDM-ACW, and BDM-mucin were carried out after 72 h of incubation at 37°C. A further experiment CB to isolate xylan fermenters used the 10^{-7} dilution of BDM-xylan, which was the top dilution showing growth after 24 h of incubation. Two experiments (G1 and G2) were also carried out in triplicate BDM-guar and SM10 (2) to 10^{-13} dilutions to establish guar utilizers as a proportion of total anaerobes, and further purifications were carried out from both the G1 10^{-10} dilution of BDM-guar after 7 days of incubation and the G2 10^{-9} dilution after 3 days of incubation.

Isolation and characterization of anaerobes. All of the colonies from the top dilutions showing growth on BDM-xylan, pectin, and arabinogalactan plates in experiment SC were subcultured into SM10 slopes. TP, G1, and G2 dilutions were streaked onto VLhlf, and after 72 h all morphologically different colonies were subcultured into SM10 slopes. Anaerobic dilution solution dilutions from the experiment CB 10^{-7} dilution were plated onto VLhlf and BDM-xylan to obtain xylan fermenters as a proportion of the total population; after 5 days of incubation representative single colonies from the top dilution of the defined agar were subcultured into SM10 slopes. Cultures were purified if necessary and then retested in BDM plus original isolation substrate to confirm that the isolates were polysaccharide fermenters. Young cultures grown for 1 or 2 days in SM10 agar were then examined by Gram stain (6) and as wet films with phase-contrast microscopy. Isolates were maintained in SM10 agar or in slopes of the appropriate defined medium, and stocks were maintained by freeze-drying. The organisms were tested for the presence of spores by heating at 70°C as described previously (5) and for aerobic growth by plating on heart infusion (Difco Laboratories) agar and incubating for 24 h at 37°C. Acid fermentation products from glucose (0.5%, wt/vol) in basal SM10 or the PY medium of Holdeman et al. (10), where stated, were determined by gas-liquid chromatography (3) with an extraction procedure (5) or a headspace analysis method. For headspace analysis, volatile free fatty acids salted out from a 1-ml sample of spent medium with 1.5 g of $MgSO_4$ and 200 μl of H_2SO_4 (50% wt/vol) were analyzed on a 3-m by 2-mm (inner diameter) glass column packed with 10% (wt/vol) SP-1000–1% (wt/vol) H_3PO_4 on 00/120 mesh Chromosorb W-AW. The column was installed in a Perkin-Elmer Sigma 1 analyzer fitted with the HS6 semiautomatic headspace sampling system. Nitrogen was used as the carrier gas at 70 ml/min, and the column was operated isothermally at 135°C. Identification procedures which followed were those of the *Anaerobe Laboratory Manual* (10). In addition, decarboxylation of glutamic acid was tested by the method of Suzuki et al. (26), and stimulation of growth by bile was tested by the method of Barnes (1) or, where stated, in SM10–20% bile (Oxgall, Difco), with estimation of stimulation or inhibition on the basis of E_{680} measurements after 24 h of incubation at 37°C.

RESULTS

Characterization of isolates utilizing pectin. Seven strains were isolated from the highest dilutions showing growth on pectin after direct plating from fecal sample SC. P1 was indole negative and could not ferment inositol, but was stimulated by the presence of bile. The isolate was unable to decarboxylate glutamic acid, but in all other respects it was characteristic of *Bacteroides distasonis*. P2 was a branching rod that most closely resembled strains of *Bifidobacterium infantis* as described by Mitsuoka (19) (Table 2). P3 was stimulated by the presence of bile and in sugar utilization pattern resembled *Bacteroides ovatus*, although it was unable to decarboxylate glutamic acid and its fermentation end acids were atypical (Table 2). P4, P5, and P6 were similar to P3. P7 differed from P1 in that it produced indole from typtophan, only weakly fermented salicin, and was probably a strain of *B. ovatus*.

Characterization of isolates utilizing xylan. Nine isolates were recovered from two xylan experiments. From experiment SC, X1 was not maintained, but had morphology and end acids from glucose similar to those of X2 and X3. Both of these isolates fermented xylose and had properties similar to those of P3, which was one of the pectin fermenters resembling *B. ovatus*. X2/1 and X2/2, isolated from enrichment experiment CB, were similar in both cell morphology and biochemical properties. On isolation both organisms grew in a palisade structure of pointed ended rods in unstirred liquid medium, although this property was lost on sub-culture. X2/1 also showed the ability to attach to surfaces when grown in a stirred fermentor, producing strands of cells held together by what appeared on microscopic examination to be extracellular material. Both strains were unable to ferment trehalose and salicin, and their reactions resembled those of *Bacteroides uniformis*, but they were not stimulated by bile. X2/3 produced acid from raffinose, starch, and trehalose, but not from salicin. It did not ferment arabinose, lactose, maltose, rhamnose, or sucrose and was therefore similar to, but atypical of, strains of *Bacteroides thetaiotaomicron* (Table 2). Of particular interest was its inability to ferment xylose in defined or complex medium unless xylan was also present as a substrate.

Characterization of isolates utilizing arabinogalactan. Of six isolates from the top dilutions showing growth on arabinogalactan from fecal sample SC, four were characterized in

TABLE 2. Characterization of fiber-fermenting isolates

Fecal sample	Isolate	Morphology	End acids from glucose[a]		Fermentation[b]																							Other biochemical tests						Possible identification
			SM10–glucose	PY-glucose	Arabinose	Cellobiose	Esculin pH	Esculin hydrolysis	Fructose	Galactose	Glucose	Inositol	Lactose	Maltose	Mannitol	Mannose	Melezitose	Raffinose	Rhamnose	Salicin	Sorbitol	Starch pH	Starch hydrolysis	Sucrose	Trehalose	Xylose	Bile[c]	Indole	Catalase	Glutamate decarboxylase	Nitrate reduction	Gelatin liquefaction	
SC	P2	Gram-positive, round-ended, branching rods; singles, pairs, and chains; 2.0 to 3.0 by 1.0 μm; nonmotile	AL (3:1)		−	−	−	−ve	a	a	a	−	a	a	−	−	−	a	−	−	−	−	−ve	a	−	a	−	−ve	−ve	−	−	−	*Bifidobacterium infantis*
SC	P3[d]	Gram-negative, round-ended rods; 2.0 to 4.0 by 1.0 μm; nonmotile	AP(b)(l)S		a	a	w	+ve	a	a	a	−	a	a	−	a	a	a	a	a	−	a	+ve	a	a	a	S	+ve	+ve	−ve	−ve	−	*Bacteroides ovatus*
CB	X2/3	Gram-negative, round-ended rods; 1.5 to 2.5 by 0.7 μm; nonmotile	ApLs		−	w	w	+ve	a	a	a	−	−	−	−	a	−	a	−	−	−	a	−ve	−	a	−	S	+ve	−ve	−	−	−	*Bacteroides* sp.
SC	A1	Gram-negative, round-ended rods; singles and pairs; 2.0 by 0.8 μm; nonmotile	AP(l)S		a	a	−	+ve	a	a	a	−	a	a	−	a	−	a	w	−	−	a	−ve	a	−	a	+[e]	+ve	+ve	−	−	−	*Bacteroides uniformis*
SC	A4[f]	Gram-positive, round-ended rods; pairs and singles; 1.1 by 4 μm; nonmotile	ABls		−	−	−	+ve	w	w	w	−	−	w	−	(w)	−	w	−	−	−	−	−ve	−	−	−	−	−ve	−ve	−	−	−	*Eubacterium* sp.
TP	B2	Gram-negative, round-ended rods; 1.5 by 2.0 by 1.0 μm; nonmotile	APLs		a	a	−	+ve	a	a	a	−	a	a	−	a	w	a	−	−	−	a	+ve	a	−	a	I[g]	+ve	−ve	−	−	−	*Bacteroides oris*
TP	B5	Gram-positive, round-ended rods with terminal spores; 2.0 by 1.0 μm, but occasionally up to 10 μm long; nonmotile	ABLs	abL	−	a	−	+ve	a	a	a	−	a	a	−	a	−	−	−	−	−	−	−ve	a	−	−	−	−ve	+ve	−	−	−ve	*Clostridium paraputrificum*

TP	ACW1	Gram-negative, round-ended rods; 2.0 by 1.0 μm, but occasionally filamentous; nonmotile	AP(b)Ls	AplS	a	a	w	+ve	a	a	a	–	a	a	–	a	–	–	–	w	–	a	+ve	a	a	a	I	+ve	–ve	–ve	–	–	*Bacteroides* sp.
G1	G1/1[h]	Gram-negative, round-ended rods; 1.5 to 2.0 by 1.0 μm; nonmotile	Ap(b)(ib) (iv)(v) (c)S		a	a	w	+ve	a	a	a	–	a	a	–	a	–	a	–	a	–	a	+ve	a	w	a	S	+ve	–ve	–	–	–	*B. ovatus* (but atypical)

[a] Capital letters indicate ≥10 μmol/ml; lowercase letters indicate ≥1 < 10 μmol-ml. Products in parentheses were present in trace amounts. Products (acids): a, acetic; p, propionic; b, butyric; ib, isobutyric; iv, isovaleric; v, valeric; c, caproic; l, lactic; s, succinic.
[b] Fermentation characters were assessed as described in the text. Abbreviations: a, pH < 5.5; w, pH 5.5 to 6.0; –, pH > 6.0 (1); +ve, positive, –ve, negative.
[c] Abbreviations: +, growth in both control and test plus 20% bile, but no stimulation; S, stimulation of growth in the presence of bile; I, inhibition of growth in the presence of bile; –, not tested.
[d] Characteristics of P4, P5, and P6 are similar to those of P3.
[e] Stimulation tested with SM10–bile.
[f] A4 was similar to A5 in terms of morphology and end acids (A5 not further described).
[g] Also tested in SM10–bile.
[h] Characteristics of G1/2 and G1/3 are similar to those of G1/1.

detail. A1 differed from other SC *Bacteroides* spp. in that it did not ferment trehalose and salicin (Table 2). It grew moderately well in the presence of bile and was tentatively identified as *B. uniformis*. A2 was a gram-positive, round-ended rod with properties very similar to those of the pectin fermenter P2 (Table 2). A3 was biochemically very similar, except that it produced acid from mannose. Apart from minor differences in cell morphology and end acids produced from glucose, all of these three most closely resembled *B. infantis* as described by Mitsuoka (19). A4 and A5 were weakly fermentative, gram-positive rods that produced acetic (14 μmol/ml); *n*-butyric (18 μmol/ml); lactic (9 μmol/ml), and succinic (3 μmol/ml) acids from fermentation of basal SM10 with glucose. End acids other than succinic acid from basal SM10–glucose are usually two- to fivefold higher than from PY-glucose, but these levels were still considerably higher than those quoted for *Eubacterium tortuosum* and *Eubacterium dolichum* (20), to which A4 otherwise showed some similarity (Table 2). The morphology for these species is also markedly different from that of A4 and A5.

Characterization of TP isolates utilizing bran. B1 was one of a group of organisms isolated as examples of distinct colony-cell morphology from the highest (10^{-10}) serial dilution of BDM-bran showing growth and was therefore probably a dominant strain within the population. It produced succinic acid as a major acid from glucose fermentation in PY; growth was stimulated in the presence of bile, but B1 was unable to decarboxylate glutamate. The fermentation pattern was characteristic of *B. uniformis*. B2, which was isolated from the 10^{-9} dilution, was inhibited in the presence of bile. It produced in excess of 20 μmol of propionic acid per ml from glucose and was indole positive, but other reactions were characteristic of *Bacteroides oris* (11) (Table 2). Isolates B3 and B4 were also from the 10^{-9} dilution and were probably strains of *B. thetaiotaomicron*. B3 was stimulated by the presence of bile, was glutamate decarboxylase negative, and, like a minority of previously described strains of *B. thetaiotaomicron* (10), it was unable to ferment cellobiose. Unlike B3, B4 was able to decarboxylate glutamate and could utilize rhamnose as a substrate. In contrast to the organisms isolated from the highest dilutions, B5 (10^{-8} dilution) was a gram-positive, sporing rod (Table 2). The fermentation pattern was similar to that of *Clostridium paraputrificum*, although B5 did not utilize starch and salicin.

Characterization of TP isolates utilizing ACW. Five isolates with differing colony morphologies were recovered from the enrichment isolation by using ACW. Isolate ACW1 from the 10^{-10} dilution appeared to be a *Bacteroides* sp. with unusual properties (Table 2). It did not produce black-pigmented colonies on blood agar. ACW2 and ACW3 were isolated from the 10^{-9} dilution; ACW2 was stimulated by bile and could decarboxylate glutamic acid, and, although the final pH of the PY-glucose fermentation was >5.5, the isolate most closely resembled descriptions of *B. thetaiotaomicron*. Growth of ACW3 was stimulated in the presence of bile; although it could not hydrolyze starch and, like a minority of strains, ferment salicin (10), its properties were generally similar to those of *B. uniformis*. Two further isolates with differing colony morphologies were recovered from the 10^{-8} dilution. ACW4 had properties similar to those of ACW2, except that it was negative for acid production from starch. ACW5 was not stimulated by bile, and it did not produce acid from esculin and salicin, but its properties were still generally similar to those of *B. uniformis*.

Characterization of isolates utilizing guar. All of the guar-

fermenting isolates grew poorly on solid media. In the top dilution showing growth in experiment G1, only one colony morphology was recovered. Three isolates, G1/1, G1/2, and G1/3, were found to have identical properties (Table 2). They are probably atypical strains of *B. ovatus* and different from the SC isolates in that they did not produce acid from rhamnose, nor, like the SC isolates, did they produce lactic acid as an end product of glucose metabolism. All of the G1 isolates produced large quantities of CO_2 when guar was the substrate. In experiment G2, three isolates were successfully maintained and characterized. G2/1 was similar to the G1 isolates in its properties, but it produced acid from esculin, trehalose, and rhamnose, and was therefore typical of *B. ovatus*. G2/2 and G2/3 had identical properties, except that the growth of G2/3 was slightly inhibited in the presence of bile. They did not produce acid from trehalose and closely resembled descriptions of *B. uniformis*. The G2 strains also produced large quantities of CO_2 from guar.

Characterization of TP isolates utilizing mucin. The enrichment isolation for mucin fermenters resulted in 14 distinct types as judged by colony morphology. Of these, five isolates were fully characterized (Table 3). M1 and M2 were weakly fermentative, gram-positive rods that did not fit published descriptions of *Eubacterium* spp. or *Actinomyces* spp. M3 produced marked amounts of gas from carbohydrates and an acetic/lactic acid ratio from basal SM10–glucose of about 3:1, together with small amounts of propionic and butyric acid. Its properties resembled those of *Bifidobacterium* spp., but were different from the SC isolates. Although like *Eubacterium* spp. on the basis of morphology and end acids, M4's other characteristics differed. M5 was originally isolated as a curved rod, but this property was rapidly lost on subculture. It had a swarming colony morphology on VLhlf, and its fermentation characteristics resembled those of *Clostridium septicum*, although spores were not detected by heating at 70°C and it did not liquefy gelatin. Conversely, M5 also resembled *Eubacterium moniliforme*, although no lactic acid was produced during fermentation of glucose.

Ability of polysaccharide fermenters to use other substrates and to attach to surfaces. The results of an experiment with TP isolates designed to show the range of substrate utilization are shown in Table 4. Growth was detected by microscopic examination. Of those isolated in bran, B3 fermented ACW, pectin, mucin, galactose, arabinose, and xylose, whereas at the other extreme, B5 only fermented bran and galactose. From the ACW isolates, ACW2 utilized all of the substrates tested, whereas three others failed to ferment pectin and mucin. The mucin fermenters had a very restricted ability to utilize complex polysaccharides and their constituent monosaccharides. Only two of the isolates were able to use fucose, which is a major component of mucin. Of the SC isolates, only one of the arabinogalactan fermenters (A6, a gram-positive, nonsporing rod that was not fully characterized) was able to utilize mucin. It is apparent, therefore, that the ability to utilize one polysaccharide substrate is not related to the ability to use others and that one substrate can be fermented by quite different organisms. Most of the isolates showed strong adherence to glass surfaces on isolation, although this property was rapidly lost on subculture.

DISCUSSION

The results reported here show that within the human fecal flora there is a specific group of bacteria capable of fermenting mucin. Although this was reported previously by Miller and Hoskins (18), the organisms involved were not characterized. In addition, our study demonstrates the existence of other groups with specific abilities. Organisms capable of the utilization of arabinogalactan were predominant in the SC isolation, which might be expected since breakdown of this substrate to monosaccharides only requires relatively simple enzymic cleavages, whereas pectin and xylan breakdown, which requires more complex cleavages, was less common. Of the pectin and xylan fermenters from experiment SC, eight of nine isolates from the top dilutions were identified as strains of *Bacteroides* spp. The xylan fermenters from experiment CB were also identified as *Bacteroides* spp., although they were different from the SC isolates. However, the SC arabinogalactan fermenters were mainly *Bifidobacterium* spp. and *Eubacterium* spp., which suggests that these genera were probably predominant among the polysaccharide fermenters within that fecal population. All of the SC isolates were tested for the utilization of mucin (by measurement of the decrease in medium pH and microscopic demonstration of growth), and only one arabinogalactan isolate was found to be able to utilize undegraded mucin for growth. The TP isolation showed that *Bacteroides* spp. have the capacity for mucin breakdown, and this ability is more common in the fecal population (for example, strain B1) than previous data would suggest (18, 21). The fact that no strains resembling B1 or B3 were actually isolated from the mucin enrichment may reflect the different affinities of the organisms for the substrate under test and may result in *Bacteroides* spp. being overgrown by the mucin enrichment isolates in defined media. The isolation of strains such as B5, which was only able to utilize a narrow range of complex and monosaccharide substrates, suggests that there are species with very specific niches in small numbers within the microbial community. The mucin fermenters were also a very distinct group, different from both the other TP isolates and from experiment SC, suggesting that enrichment techniques can permit recovery from the fecal community of isolates with a range of very distinct properties. Their extremely restricted range of substrate utilization indicates that the presence of plant polysaccharides in the human colon has little protective effect against the use of colonic mucin as an energy source. Many of the mucin fermenters were difficult to maintain despite the use of strict anaerobic techniques. This may reflect a requirement for specific growth conditions, such as high viscosity, which may mimic the in vivo wall-associated niche or, alternatively, a need for other substrates such as cell membrane glycolipids and proteins. The mucin fermenters did not closely resemble previous descriptions in the literature, nor were they similar to strains shown by Salyers et al. to ferment mucin (21, 22). This may be due to the use of enrichment techniques for the isolation, leading to the recovery of organisms masked in a conventional isolation with complex media. Some of the organisms isolated from the guar enrichments, although identified as *Bacteroides* spp., are different from strains described previously as fermenting guar in a survey (21) in which 4 of 24 strains of *B. ovatus* and 17 of 19 strains of *Bacteroides* sp. 0061-1 (now identified as *B. uniformis* [15]) were found to be positive. Complete breakdown of guar is thought to be dependent on the presence of β-mannanase (to cleave the mannose backbone), an α-galactosidase (to remove galactose side chains from the backbone), and a β-mannosidase to accomplish the final step to monosaccharides. Although β-mannosidase activity could be detected in fecal incubates, it was not found in any of the guar-fermenting isolates tested in pure culture, suggesting that other organisms in the mixed culture may be responsible for the complete degradation of

TABLE 3. Characterization of TP isolates fermenting mucin[a]

Isolate	Morphology	End acids from glucose (μmol/ml)	Fermentation																						Other biochemical tests		Possible identification
			Arabinose	Cellobiose	Esculin pH	Esculin hydrolysis	Fructose	Galactose	Glucose	Inositol	Lactose	Maltose	Mannitol	Mannose	Melezitose	Raffinose	Rhamnose	Salicin	Sorbitol	Starch pH	Starch hydrolysis	Sucrose	Trehalose	Xylose	Indole	Catalase	
M1[b]	Gram-positive, round-ended rods; pairs and long chains; 1.5 to 3.5 by 0.5 to 0.75 μm; nonmotile	AblS (90:1:6:36)	−	−	−	−ve	−	w	w	−	−	−	−	w	−	−	−	−	−	−	−ve	−	−	−	+ve	−ve	Unknown
M3	Gram-positive, round-ended rods; singles and pairs; 1.5 to 3.0 by 0.75 to 1.0 μm; nonmotile	A(p)bL (69:trace:1:20)	a	w	−	+ve	a	a	a	−	a	w	−	−	−	−	−	−	−	−	−ve	a	a	−	−ve	−ve	*Bifidobacterium* sp.
M4	Gram-positive, round-ended rods; 1.5 to 3.0 by 0.8 to 1.0 μm; nonmotile	Apbl (72:3:2:4)	w	−	w	+ve	−	a	a	−	a	a	−	−	−	a	−	a	−	−	−ve	a	a	−	−ve	−ve	Unknown
M5	Gram-positive, square-ended rods with vacuoles; 1.5 to 4.0 by 1.0 μm; nonmotile	ApBs (33:2:55:4)	−	−	−	−ve	a	w	a	−	a	a	−	a	−	−	−	−	a	−	−ve	−	−	−	−ve	+ve	Unknown

[a] Abbreviations and symbols as in Table 2.
[b] M2 was identical to M1, except M2 was catalase positive.

the polysaccharide and that guar utilizers may use another method for the breakdown of mannose oligosaccharides. The ability of many of the isolates to adhere to surfaces during growth and of some of the xylan fermenters to grow in palisades and the appearance of X2/1 when grown in a stirred system are all important characteristics, since the ability to adhere to, and multiply on, a surface or layer against the flow of material through the colon must be important survival mechanisms for gut anaerobes.

The work reported here has established that many organisms inhabiting specific substrate niches within the fecal population remain to be described, and that experiments on dietary fiber utilization carried out with strains from total fecal isolations on nonselective media do not reflect the situation that pertains in vivo. The isolations demonstrate both the independence and interspecies dependence of the bacteria within the microbial community of the human colon. Further experiments will be required to demonstrate how fermentation of polysaccharide substrates by mixed cultures, as opposed to single isolates, affects enzyme induc-

TABLE 4. Ability of TP isolates to grow in a range of substrates

Isolate	Growth in substrate							
	ACW[a]	Pectin[a]	Bran[a]	Mucin[a]	Fucose[a]	Galactose[b]	Arabinose[b]	Xylose[b]
B1	+	−	+[c]	+	NT[d]	+	+	+
B2	−	−	+[c]	−	NT	+	+	+
B3	+	+	+[c]	+	NT	+	+	+
B4	+	+	+[c]	−	NT	+	+	+
B5	−	−	+[c]	−	NT	+	−	−
ACW1	+[c]	−	+	−	NT	+	+	+
ACW2	+[c]	+	+	+	NT	+	+	+
ACW3	+[c]	−	+	−	NT	+	+	+
ACW4	+[c]	+	+	+	NT	+	+	+
ACW5	+[c]	−	+	−	NT	+	+	+
M1	−	−	−	+[c]	−	+	−	−
M2	−	−	−	+[c]	−	+	−	−
M3	−	−	−	+[c]	+	+	+	−
M4	−	−	−	+[c]	+	+	−	−
M5	−	−	−	+[c]	−	+	−	−

[a] Substrate in BDM at 0.2% (wt/vol).
[b] Substrate in PY medium (10) at 0.5% (wt/vol).
[c] Original isolation medium.
[d] NT, Not tested.

tion and the short-chain fatty acid end products that are important in providing energy for the epithelial cells of the human colon.

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