Cellulolytic and Non-Cellulolytic Bacteria in Rat Gastrointestinal Tracts

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Lactobacillus and Bifidobacterium species were the predominant organisms isolated from small intestinal (jejunal) contents of rats, and lactic acid was the only organic acid detected. The numbers of cellulolytic bacteria in small intestines were low (approximately 10^3/g). The fermentation in ceca was different from that in intestines, as, in addition to small amounts of lactic acid, high concentrations of volatile fatty acids were detected. The mixed cecal microflora was able to digest cellulose (pebble-milled Whatman no. 1) and cabbage. High numbers of cellulolytic bacteria were found (0.5 × 10^9 to 12.2 × 10^9/g; 6% of total viable bacteria). The predominant cellulolytic organism isolated was Bacteroides succinogenes. Ruminococcus flavigaliens was isolated from a few animals. The kinds and numbers of the predominant non-cellulolytic organisms isolated from rat ceca were similar to those described by previous workers.

The presence of dietary fiber in the diet is correlated with lower incidences of coronary heart disease, arteriosclerosis, colonic cancer, gallstones, diverticular disease, and diabetes mellitus (35, 36). Since dietary fiber consists of complex carbohydrates of plant origin that mammalian gut enzymes cannot digest, a number of investigators have suggested that the beneficial effects of fiber may be due not only to their physical or chemical nature but also to their fermentation in the cecum and colon (35). To determine the extent of fiber degradation in the lower gut, we decided to attempt the enumeration and isolation of cellulolytic bacteria from the rat cecum. Emphasis was placed on cellulolytic bacteria because cellulose is the most difficult of complex carbohydrates to degrade. If such organisms are found in significant numbers, one can conclude that dietary fiber is probably fermented in this environment.

Reports supporting the hypothesis that lower gut microflora ferment fiber resulted from work with animals having ceca and with humans. Significant numbers of cellulose-degrading organisms were found in the ceca of guinea pigs (11), rabbits (15), and horses (10). A cellulolytic organism (a Bacteroides species; 10^9/g) was isolated from one of five human fecal samples (1, 2). Additionally, van Soest et al. (39, 40) and Ehle et al. (12a) demonstrated that the mixed microbial populations from human feces can degrade dietary fibers such as bran and cabbage. The presence of bran or cabbage in the diet did not significantly affect the ability of fecal organisms to degrade various kinds of fiber (12a). Salyers and co-workers found that human colonic Bacteroides sp. can ferment a variety of complex polysaccharides, including xylans, non-cellulosic glucans, pectins, galactomannans, arabinogalactans, mucopolysaccharides, and mucin glycoprotein (20, 30, 31). Finally, Chang and co-workers observed that the addition of bran or corn bean to a diet increased the activities of β-glucosidase (2.5-fold) and α-galactosidase (4-fold) in human feces (30; G. W. Chang, H. E. Fukumoto, C. P. Gyory, A. P. Block, M. J. Kretsch, and D. H. Calloway, Fed. Proc. 38:767, 1979; G. W. Chang, personal communication).

MATERIALS AND METHODS

Animals and diets. Male Sprague-Dawley rats were obtained from the Department of Animal Science Small Animal Colony, University of California, Davis. Immediately after weaning (3 weeks old), the rats were fed a stock diet (Ralston Purina Co., St. Louis, Mo.; crude fiber, "no more than 6%") Food and water were available ad libitum. Rats used for experiments were 6 to 8 weeks old (average age, 7 weeks).

Intestinal sampling and processing. Rats were anesthetized with ether, and the intestines were exposed via a midventral incision. The duodenal and ileal ends of the small intestine and the opening to the cecum were ligated. The different areas of the intestine were separated from each other and placed in ice. The small intestine and cecum were transferred to an anaerobic chamber (Coy Laboratory Products, Inc., Ann Arbor, Mich.). The contents of the middle portion (15 to 20 cm) of the small intestine (jejunum) or the entire contents of the cecum were emptied into small beakers held on ice. After thorough mixing of the contents with

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TABLE 1. Microbiological media

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>PY-CF broth</th>
<th>PY-CF agar</th>
<th>PY-CF-MS broth</th>
<th>PY-CF-MS agar</th>
<th>Dilution broth</th>
<th>PMC broth</th>
<th>PMC agar</th>
</tr>
</thead>
<tbody>
<tr>
<td>Solution A</td>
<td>167 ml</td>
<td>167 ml</td>
<td>167 ml</td>
<td>167 ml</td>
<td>167 ml</td>
<td>167 ml</td>
<td>167 ml</td>
</tr>
<tr>
<td>Solution B</td>
<td>167 ml</td>
<td>167 ml</td>
<td>167 ml</td>
<td>167 ml</td>
<td>167 ml</td>
<td>167 ml</td>
<td>167 ml</td>
</tr>
<tr>
<td>Peptone</td>
<td>5.0 g</td>
<td>5.0 g</td>
<td>5.0 g</td>
<td>5.0 g</td>
<td>5.0 g</td>
<td>5.0 g</td>
<td>5.0 g</td>
</tr>
<tr>
<td>Yeast extract</td>
<td>10.0 g</td>
<td>10.0 g</td>
<td>10.0 g</td>
<td>10.0 g</td>
<td>10.0 g</td>
<td>10.0 g</td>
<td>10.0 g</td>
</tr>
<tr>
<td>Trypticase (BBL)</td>
<td>5.0 g</td>
<td>5.0 g</td>
<td>5.0 g</td>
<td>5.0 g</td>
<td>5.0 g</td>
<td>5.0 g</td>
<td>5.0 g</td>
</tr>
<tr>
<td>NaHCO₃</td>
<td>5.0 g</td>
<td>5.0 g</td>
<td>5.0 g</td>
<td>5.0 g</td>
<td>5.0 g</td>
<td>5.0 g</td>
<td>5.0 g</td>
</tr>
<tr>
<td>Cysteine-HCl</td>
<td>0.25 g</td>
<td>0.25 g</td>
<td>0.25 g</td>
<td>0.25 g</td>
<td>0.25 g</td>
<td>0.25 g</td>
<td>0.25 g</td>
</tr>
<tr>
<td>Na₂S · 9H₂O</td>
<td>0.25 g</td>
<td>0.25 g</td>
<td>0.25 g</td>
<td>0.25 g</td>
<td>0.25 g</td>
<td>0.25 g</td>
<td>0.25 g</td>
</tr>
<tr>
<td>Vitamin K₃</td>
<td>0.001 g</td>
<td>0.001 g</td>
<td>0.001 g</td>
<td>0.001 g</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hemin</td>
<td>0.0025 g</td>
<td>0.0025 g</td>
<td>0.0025 g</td>
<td>0.0025 g</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sugar mixture</td>
<td></td>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Agar</td>
<td>15.0 g</td>
<td>15.0 g</td>
<td></td>
<td>5.0 g</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intestinal-cecal extract</td>
<td>100 ml</td>
<td>100 ml</td>
<td>100 ml</td>
<td>100 ml</td>
<td>100 ml</td>
<td>100 ml</td>
<td>100 ml</td>
</tr>
<tr>
<td>PMC</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>6.7 g</td>
<td></td>
<td>5.0 g</td>
</tr>
<tr>
<td>Vitamin mixture</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>VFA mixture</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

a Final volume, 1 liter.

b Solution A contained (in grams per liter): NaCl, 5.4; KH₂PO₄, 2.7; CaCl₂ · 2H₂O, 0.159; MgCl₂ · 6H₂O, 0.12; MnCl₂ · 4H₂O, 0.06; CoCl₂ · 6H₂O, 0.06; (NH₄)₂SO₄, 5.4.

c Solution B contained (in grams per liter): K₂HPO₄, 2.7.

d Prepared as a cysteine · HCl-Na₂S solution under an atmosphere of 100% N₂ and autoclaved separately (22).

e A mixture of the following sugars (each 5%) was prepared anaerobically under 100% N₂ and autoclaved separately: glucose, maltose, cellobiose, xylose, fructose. Final concentration of each sugar was 0.2%.

f Indicates that this mixture was added.

x Oxid purified agar.

h The extract ("cecal") extract of rat intestinal and cecal contents (from ceca and intestines stored at ~20°C) was prepared under an atmosphere of 100% CO₂ by extracting 1 part contents with 2 parts water in a Waring blender. The suspension was centrifuged at 17,000 × g for 1 h at 4°C; the supernatant was transferred, under an atmosphere of 100% CO₂, to roll tubes and autoclaved.

i The PMC was prepared by pebble milling 20 g of Whatman no. 1 filter paper in 1 liter of distilled water for 16 to 17 h. This suspension was then autoclaved and stored at 4°C until use.

j The vitamin mixture added was that of Scott and Dehorthy (32).

k The VFA mixture was that used by Caldwell and Bryant in medium 10 (16), but at a fivefold lower concentration.

Media for enumeration, isolation, and characterization of bacteria. All media were prepared anaerobically under an atmosphere of 100% CO₂ according to the method of Hungate (18). The medium used to enumerate and isolate viable bacteria from either the small intestine or cecum of rats was the peptone-yeast extract-mixed sugars-"cecal fluid extract" (PY-CF-MS) agar medium described in Table 1. This medium is similar to the PY medium of Holdeman et al. (16), except that the mineral solution used was that of Varel and Bryant (41), and an extract of intestinal plus cecal contents was present.

The medium described above was chosen because it simulates the conditions in the rat intestine and cecum. Preliminary experiments comparing this medium with the same medium to which a vitamin and VFA solution (Table 1) had been added or with a rumen fluid medium (18) (in which the peptone, yeast extract and "cecal fluid" of the PY-CF-MS medium were replaced by 30% rumen fluid) showed that there was no significant difference in numbers of organisms detected.

Dilution medium and the medium used for the enumeration of cellulolytic bacteria are shown in Table 1.

Cellulolytic organisms were isolated in a pebble-milled cellulose (PMC) agar medium (Table 1). This medium contained only 0.5% agar, thus allowing clear zones to form. To permit simple observation of clearings that formed, the cellulose concentration was 0.5%, and only 3 ml of medium was added to each 1.6-by-15-cm roll tube.

Organisms were characterized by using a PY-CF...
brook medium to which the appropriate substrate was added (Table 1).

Enumeration and isolation of bacteria. After inoculation, rolling, and incubation of the enumeration medium, numbers of each colony type were determined in tubes containing 30 to 300 colonies. Representative colonies of each type were then purified according to the method of Hungate (18) by subculturing several times in PY-CF-MS agar medium. Pure cultures were grown in PY-CF-MS broth medium and lyophilized. In the preparation of cultures for lyophilization, all manipulations were carried out in the anaerobic chamber. Organisms were identified according to the procedures of Holdeman et al. (16) with PY-CF agar and broth media instead of PY medium. We also referred to Bergey's Manual of Determinative Bacteriology (7).

Numbers of cellulolytic organisms were determined by using the most-probable-number (MPN) technique (9) in PMC broth medium, because numbers estimated with PMC agar medium were erratic. Three tubes of the PMC broth medium were inoculated with 0.5 ml of cecal contents serially diluted 10-fold. After several transfers of the highest dilution of MPN cultures showing cellulose disappearance in PMC broth medium, cellulolytic organisms were isolated from the enrichments by first diluting (18) them in PMC agar medium. Organisms forming clear zones were purified by subculturing them several times in PMC agar. Cellulolytic organisms were lyophilized and identified as described above.

In vitro fermentation studies. Media used for in vitro fiber fermentation studies were prepared anaerobically under 100% CO₂ (18) and had the following composition: inorganic salts and reducing agent, as for all media; a freshly prepared extract of intestinal-cecal contents, 33%; and one of the following substrates: 1.0% ethanol-extracted cabbage, 0.5% PMC, or no substrate (control). For each substrate, 90 ml of medium was prepared in a 250-ml round-bottom flask. The medium was not autoclaved. Ethanol-extracted cabbage was prepared by a method similar to that of Ehle et al. (12a); the cabbage was soaked in 95% ethanol (four changes at approximately 1-h intervals), dried overnight, and then ground in a Wiley mill with a 20-mm mesh screen.

To determine the extent of fiber degradation by mixed cecal organisms in vitro, the ceca from four rats were placed in the anaerobic chamber, and their contents were pooled. The contents were weighed, and 2 volumes of dilution medium were added. This mixture was thoroughly homogenized with a Virtis "45" homogenizer. Equal volumes of the suspension were added to duplicate fermentation vessels containing the different fiber substrates. The average inoculum size for all experiments was 2.9% (wt/vol; range, 2.35 to 3.15%). Flasks were removed from the anaerobic chamber, the atmosphere was changed to 100% CO₂, and the flasks were incubated in a 37°C water bath for 48 h. Digestibility of cell wall fibers in the suspensions was determined (12a).

Analysis of fermentation products. Hydrogen was analyzed at room temperature with a Perkin-Elmer 154B gas chromatograph (The Perkin-Elmer Corp., Norwalk, Conn.) with a silica gel column and N₂ as the carrier gas. Formic acid was analyzed by the method of Lang and Lang (21). All other VFA were measured with a Varian model 3700 gas chromatograph (Varian Associates, Inc., Palo Alto, Calif.) equipped with a flame ionization detector under the following conditions: stainless steel column (6 ft by 0.125-in. outer diameter; 0.093-in. inner diameter [ca. 2 m by 3.2 mm; 2.4 mm]) packed with 15% FFAP chromosan acid wash; injector, 200°C; oven, 144°C; detector, 219°C; carrier gas, argon at a flow rate of 16 psig. D- and L-lactic (28) and succinic (24) acids were estimated enzymatically.

RESULTS

Gut fermentation. Organic acids were found in both the small intestines (jejuna) and ceca of rats (Table 2), indicating that fermentation occurs in the gastrointestinal tracts of rats, as found previously (12). The fermentation in the small intestine appeared to be homolactic, as significant amounts of lactic acid, but no VFA, were detected. The fermentation in the cecum yielded large amounts of acetic, propionic, and butyric acids; some lactic acid was also present, but in concentrations lower than those found in the small intestine.

Fiber degradation by mixed cecal flora. To determine whether cecal microflora are capable of degrading dietary fiber, cecal contents were inoculated into anaerobic media containing PMC or cabbage. Both of these fiber sources were fermented by the mixed cecal population. Cube fermentation was significantly greater (Table 3).

<table>
<thead>
<tr>
<th>Site</th>
<th>Acetic</th>
<th>Propionic</th>
<th>Butyric</th>
<th>Lactic</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intestine</td>
<td>&lt;0.5</td>
<td>&lt;0.3</td>
<td>&lt;0.2</td>
<td>18.3 ± 5.9</td>
</tr>
<tr>
<td>Cecum</td>
<td>71.7 ± 12.2</td>
<td>32.2 ± 4.8</td>
<td>16.8 ± 3.6</td>
<td>5.9 ± 1.0</td>
</tr>
</tbody>
</table>

* Average from six animals sampled on different days. Values are expressed as micromolar concentration ± standard error of the mean; n = 6. Percent water of cecal contents was 80.3 ± 1.3.

TABLE 3. Percent cabbage and PMC digested by mixed cecal flora after 48 h of incubation

<table>
<thead>
<tr>
<th>Expt</th>
<th>Cabbage</th>
<th>Cellulose</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>68</td>
<td>36</td>
</tr>
<tr>
<td>II</td>
<td>63</td>
<td>20</td>
</tr>
</tbody>
</table>

* Inoculum: 2.9% (wt/vol) (range, 2.35 to 3.15%) of cecal contents.
Enumeration and isolation of cellulytic bacteria. After unsuccessful initial attempts (i.e., clearing of cellulose was not observed) to enumerate and isolate cellulytic degrading organisms by using a PMC agar medium with a cellulose concentration of 0.8% and an agar concentration of 1.5%, we began using PMC broth medium. The absence of agar in the medium allowed cellulose to be degraded. Initially, single tubes of PMC broth medium were inoculated with the serially diluted intestinal material. Results indicated that the number of cellulytic bacteria per gram of contents (wt weight) was approximately five orders of magnitude lower in the small intestine (approximately 10^3/g) than in the cecum of rats (data not shown). Accordingly, only the cellulytic flora of the cecum was investigated further.

The MPN of cellulolytic degrading organisms was estimated from the number of inoculated tubes in which cellulose degradation was detected. Degradation was usually visible within 8 days, but tubes showing no degradation were incubated further (up to 3 months). Cellulytic organisms accounted for approximately 6.3% of the total number of viable bacteria in the cecum of rats. The number of viable organisms was 72 × 10^3/g (average of six animals; range, 59 to 102), and the number of cellulolytic organisms was 4.53 × 10^3/g (range, 0.81 to 12.2).

Isolation of cellulytic bacteria from the cellulose enrichments was finally accomplished by first transferring the highest dilution of broth showing cellulose degradation in PMC broth medium several times. Cellulytic organisms were isolated from the enrichments by first diluting them in a PMC agar medium that contained 0.5% PMC and only 0.5% agar. The lower concentration of agar allowed visible clearing to occur. The lower cellulose concentration and medium volume (3 ml) made it easier to see the clearings. After incubation for 16 to 20 days, two kinds of cellulose degradation zones were observed in the thin cellulose agar film; organisms responsible for forming the zones were purified by transferring them several times into PMC agar medium. The kind of clear zone that appeared most frequently (in material from 16 of 21 animals) showed no visible colony. The organisms isolated from this kind of clear zone were small, gram-negative rods and were classified as Bacteroides succinogenes. The less frequently observed clear zones contained a colony (in 5 of 21 animals) that yielded gram-positive cocci in pairs or short chains. These organisms were identified as Ruminococcus flavefaciens. A description of the characterization of these two types of cellulose-degrading organisms is presented elsewhere (25).

Because the use of PMC agar medium proved so successful for isolating rat cecal cellulytic organisms from PMC broth cultures, attempts were made to enumerate and isolate cellulytic organisms directly from cecal contents by using this same medium. Although discrete clearings were detected and isolations were successful with PMC agar medium, numbers of cellulose degraders, as estimated from the number of clear zones, were erratic and slightly lower than those determined with the MPN technique. In addition, B. succinogenes strains were the only cellulose degraders isolated from these agar dilutions. This was the case even when ruminococci (and not B. succinogenes) were isolated from the MPN broth inoculated with the same diluted sample of cecal material. This may be because the ruminococci were present in such low numbers compared to the non-cellulytic organisms (non-cellulytic bacteria outnumbered cellulytic bacteria 17 to 1) that they simply could not compete with the more numerous members of the flora that intercepted products of ruminococcus cellulase hydrolysis. Thus, it appears that the MPN technique is the most reliable approach to enumeration and isolation of cellulytic organisms.

Enumeration and isolation of non-cellulytic bacteria. The numbers and kinds of predominant non-cellulytic organisms isolated from the intestinal and cecal contents of rats are shown in Tables 4 and 5. A total of 93 organisms were isolated in six different experiments (41 and 52 from intestine and cecum, respectively). Total numbers of viable organisms detected in the ceca of rats were higher than those in the small intestine. The predominant organisms isolated from the small intestine were Lactobacillus spp. and Bifidobacterium spp. The most numerous organisms detected in ceca were Lactobacillus spp., Eubacterium spp., Veillonella spp., and Bacteroides sp.

**TABLE 4. Total numbers of organisms and numbers of each genus isolated from the intestinal contents of rats**

<table>
<thead>
<tr>
<th>Organism</th>
<th>No. of organisms (×10^9/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td>8.68–56.9 (31)</td>
</tr>
<tr>
<td>Lactobacillus spp.</td>
<td>8.45–56.9 (6)</td>
</tr>
<tr>
<td>Bifidobacterium</td>
<td>0.07–5.29 (3)</td>
</tr>
<tr>
<td>Eubacterium spp.</td>
<td>0.23 (1)</td>
</tr>
</tbody>
</table>

*a Numbers shown are the ranges of six experiments.
*b Mean of six experiments.
*c Number of experiments in which organism was isolated; six experiments (i.e., six animals) were done.

DISCUSSION

The purpose of the experiments described here was to isolate one of the most important
groups of organisms involved in fiber fermentation, the cellulolytic organisms, to determine whether or not this dietary fiber is digested in the ceca of rats, as has been suggested (35). In rat ceca, cellulolytic bacteria were found in high numbers (0.5 × 10⁷ to 12.2 × 10⁷/g) with the MPN technique and accounted for 6.3% of the total organisms isolated. The predominant cellulose degrader isolated was \textit{B. succinogenes}. When human fecal material was examined by Bryant and co-workers, also with the MPN technique and pebble-milled (PM) Whatman no. 1 cellulose broth, a cellulolytic \textit{Bacteroides} sp. was found in high numbers (greater than 10⁸/g). The organism isolated, however, accounted for only 0.3% of the total viable flora and was found in only one of five individuals sampled (1, 2). Since Ehle found that all human fecal microbial populations tested were able to ferment bran (9.3% cellulose) and cabbage (31.1% cellulose) regardless of diet (12a), the major cellulolytic flora of humans most probably remains to be isolated. Perhaps the organisms could be found if cabbage cellulose was used as a substrate.

The numbers of cellulolytic organisms detected in the present study were significantly higher than those found in ceca of other animals, such as the guinea pig (approximately 10⁷/g [11]), rabbit (0.05 × 10⁷ to 7.2 × 10⁷ [15]), and horse (0.007 × 10⁹ to 1 × 10⁹ [10]). Although \textit{B. succinogenes} was the predominant cellulose-degrading organism isolated from rat ceca, \textit{Ruminococcus} sp. was the only type of cellulolytic bacteria isolated from guinea pig (11) and rabbit (15) ceca. Several types of cellulolytic bacteria have been isolated from horse ceca (similar to \textit{Ruminococcus} sp., \textit{Bacteroides} sp., \textit{Clostridium} sp., and \textit{Butyrivibrio} sp. [10]), but none of these has been identified and characterized.

We may have obtained higher numbers of cecal cellulolytic organisms in our studies than did previous workers because of the different isolation methods used. Enumeration and isolation of cellulose degraders from guinea pigs, rabbits, and horses were accomplished by inoculating diluted cecal contents directly into a cellulose medium containing normal agar concentrations (i.e., by selective isolation). In each case, the cellulose source was different (guinea pig, PM Solka Floc [11]; rabbit, PM Whatman no. 1 [15]; horse, a “fine suspension” [34] of cellulose [10]). In our experiments, selective isolation in a cellulose (PM Whatman no. 1) agar medium containing vitamins and VFA was not successful unless the agar concentration (purified agar) was lowered to 0.5%. Even then, accurate enumeration was impossible because the results obtained were erratic. The most reliable method for estimating numbers of cellulolytic organisms was the MPN technique in PMC broth medium; isolation from broth was then accomplished in PMC agar medium with 0.5% agar. Considering the high numbers of organisms isolated in our studies and the inability of our \textit{B. succinogenes} strains to degrade cellulose in PMC agar medium which contained a normal amount of agar, the concentration of agar used in selective media may have prevented isolation of the predominant cellulolytic bacteria from guinea pig, rabbit, and horse ceca.

This hypothesis is supported by the finding that the use of high concentrations of agar with PM Whatman no. 1 cellulose also made growth of rumen strains of \textit{B. succinogenes} difficult in rumen studies; this organisms was either not isolated (37, 38) or was isolated infrequently and in low numbers (17). The organism was only isolated selectively in significant numbers when acid-treated PM cotton cellulose was included in an agar medium with a normal agar concentration (13, 14, 19, 23, 33, 37). \textit{B. succinogenes} was also isolated nonselectively along with other predominant rumen bacteria when a medium designed to support growth of all rumen bacteria was inoculated from dilutions of rumen fluid (3–6, 22). One of the cellulolytic \textit{B. succinogenes} strains isolated in this nonselective manner, S85 (the neotype strain [8]), was unable to grow in a medium containing PM Whatman no. 1 cellulose (0.4 to 0.5%) and the normal concentration of agar. When the agar concentration was lowered, however, S85 was able to grow and utilize cellulose in the presence of agar (25).

Clearly, further studies must be done to optimize cellulose agar media such that all cellulose-degrading gut anaerobes will be able to grow. This would assist in the accurate enumeration of these organisms from many different environments.

The results we obtained concerning non-cellulolytic organisms isolated in this study are simi-
lar to those reported previously (26, 27, 29). The only significant differences are that we detected higher numbers of lactobacilli (26, 27, 29) than did other workers and slightly lower numbers of Bacteroides sp. than were detected in two studies (26, 27). The reason for such differences is unclear, as adequate anaerobic techniques were employed in all the studies. The results, however, may have been influenced by different media used for enumeration and isolation of the organisms in each study (26, 27, 29).

Since it is now clear that cellulose is degraded by microbes from the ceca of rats, further studies must be done to determine how the fermentation of dietary fiber in the lower gut either directly or indirectly protects the animals from contracting diseases associated with diets low in fiber (35).

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LITERATURE CITED