**Bifidobacterium lactis** Bb-12 and **Lactobacillus salivarius** UCC500 Modify Carboxylic Acid Formation in the Hindgut of Rats Given Pectin, Inulin, and Lactitol\(^1,2\)

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**Abstract**

The effect of **Bifidobacterium lactis** (Bb-12) and **Lactobacillus salivarius** (UCC500) on the formation of carboxylic acids (CAs) was studied in the hindgut of rats fed pectin, inulin of low solubility, and lactitol. When the pectin diet was supplemented with Bb-12, the formation of CAs was larger throughout the colon of rats, due to increased formation of acetic acid (\(P < 0.01\)) and, in the distal part of the colon, also because of propionic and butyric acids (\(P < 0.01\)). In rats fed pectin and UCC500, there was a shift in the formation of CAs from the cecum to the distal colon. Thus, the cecal pool of CAs in the rats was lower (\(P < 0.05\)), whereas the concentration of CAs in the distal part of colon was larger (\(P < 0.01\)) than without this strain. Concerning the slowly fermentable inulin, there was a greater formation of CAs in the cecum (\(P < 0.05\)) of rats, especially propionic acid, and a lower formation in the distal part of the colon (\(P < 0.01\)) when the diets were supplemented with Bb-12, whereas UCC500 had no effect except for a lower proportion of acetic acid in the distal part of the colon (\(P < 0.001\)). In rats fed lactitol and Bb-12, the concentration of CAs was lower in the distal part of colon (\(P < 0.001\)) than without this strain, whereas the cecal pool of CAs was greater in rats supplemented with UCC500 (\(P < 0.001\)). We conclude that the probiotic bacteria affect the amount, the pattern, and the site of release of CAs in the hindgut of rats, but the combination of pre-and probiotics is of great importance for the outcome. J. Nutr. 136: 2175–2180, 2006.

**Introduction**

Dietary carbohydrates reaching the colon, usually referred to as indigestible carbohydrates (ICs),\(^5\) are important substrates for the colonic microflora, and it was suggested that at least 30 g IC/d is required to maintain the microflora (1). In the colon, the ICs are degraded to carboxylic acids (CAs), mainly acetic, propionic, and butyric acid. Some of the CAs have important physiological functions. Butyric acid, which is the main energy source for the colonocytes, was shown to decrease the risk of mucosal damage (2). This acid has a trophic effect on the intestinal epithelium, by increasing the blood flow and stimulating mucosal proliferation. Furthermore, butyric acid appears to stimulate apoptosis in tumor cell lines, and it has increasingly been considered useful in the prevention or treatment of colonic diseases, e.g., ulcerative colitis and cancer. Propionic and acetic acid may have similar but less prominent effects. These acids are associated mainly with metabolic effects (2). Human rectal infusions of propionate and acetate showed that the elevating effect of acetate on blood cholesterol levels could be reduced by propionate, and it was suggested that the higher the propionic:acetic acid ratio, the more beneficial is the effect (3).

Different ICs were shown to give rise to different amounts and patterns of CAs in the colon depending on the solubility, degree of polymerization, type of linkages, branching, and monomeric composition (4,5). These factors may also be of importance for the site of fermentation. The complexity of the food matrix (6) and factors such as colonic pH, transit time, and the composition of the colonic microflora may also have an effect (7). A possible way to modify the composition of the microflora is to incorporate probiotic bacteria in the diet. The effects of probiotics on CA formation have not been investigated to any great extent in vivo. However, in vitro studies with pig cecal bacteria showed that the same amount of CAs was produced, but production was faster when probiotics (**Lactobacillus casei** and **Bifidobacterium breve**) were added (8). Probiotics, generally lactobacilli or bifidobacteria, are frequently used in foods and have been ascribed several health benefits, such as inhibition of the activity and growth of pathogens and stimulation of the

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\(^1\) Portions of data from rats fed the control diets (inulin and lactitol) were published previously (Nilsson U, Nyman M. Short-chain fatty acid formation in the hindgut of rats fed oligosaccharides varying in monomeric composition, degree of polymerization and solubility. Br J Nutr. 2005;94:705–13). This study was carried out with the financial support of the Commission of the European Communities, specific RTD program “Quality of Life and Management of Living Resources,” QLK1-2000-300042, “PROTECH.” It does not necessarily reflect its views and in no way anticipates the Commission’s future policy in this area.

\(^2\) Abbreviations used: Bb-12, **Bifidobacterium lactis**; CA, carboxylic acid; cfu, colony forming units; dw, dry weight; IC, indigestible carbohydrate; RAPD, randomly amplified polymorphic DNA; UCC500, **Lactobacillus salivarius**.

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immune system (9). The mechanisms behind these effects are primarily unknown but could be due at least in part to the CAs formed. Combining probiotics with fermentable carbohydrates may offer potential benefits with respect to colonic health, including modification of CAs formation and site of release.

The aim of the present study was to investigate whether CA formation is affected by probiotic bacteria when added to diets containing fermentable carbohydrates, by using a rat model. For this purpose, pectin, inulin, and lactitol were selected as the colonic substrates and *Bifidobacterium lactis* (Bb-12) or *Lactobacillus salivarius* (UCC500) as the probiotic bacteria. Bb-12, the most thoroughly studied probiotic *Bifidobacterium* strain currently on the market, was shown to be effective in preventing traveler's diarrhea, decreasing the risk of constipation, and in the modulation of the immune response (9). *Lactobacillus salivarius* has not been studied to any great extent, but some strains were shown to have anti-inflammatory effects and can exist at an extremely low pH (10,11). All of the carbohydrates selected reach the colon, are highly fermented, and are therefore potential prebiotic substrates, i.e., colonic substrates that specifically increase the number of bacteria that have beneficial physiological effects. Pectin was chosen because this polysaccharide was shown to give high proportions of acetic acid during fermentation (6), whereas the type of inulin used has a low solubility and is comparatively slowly fermented, giving high proportions of butyric and propionic acid in the distal part of the colon (5). Further, inulin was reported to stimulate the growth of bifidobacteria (12). Lactitol was selected because it was shown to give rise to surprisingly low cecal amounts of CAs during fermentation and was proposed to be rapidly fermented (5).

### Materials and Methods

#### Materials

Three commercial indigestible carbohydrate sources (pectin, inulin, and lactitol) were included in the study. Inulin (Rafitline HPX) derived from chicory root (obtained from ORAFTI), is a long-chain fructan with an average degree of polymerization of 23 (10–60). Its powder characteristics were changed during production to obtain a lower solubility (1 g/L at 25°C vs. 25 g/L at 25°C for ordinary long-chain inulin) (13). Lactitol (galactose and glucitol) and pectin were obtained from Danisco. The pectin, which was derived from citrus fruit (lime), had a degree of esterification of 70.7%. Two bacterial strains were included in the study: *Bifidobacterium lactis* (Bb-12, Chr. Hansen) and *Lactobacillus salivarius* (UCC500). The 2 strains were delivered freeze-dried and each strain was added to diets with each of the carbohydrate sources.

#### Experimental design, rats, and diets

Three control diets containing pectin, inulin, or lactitol were included. Six test diets were prepared in which UCC500 or Bb-12 was added to each of the 3 control diets. Male Wistar rats (*n* = 63), 3–4 wk old, with an initial weight of 92.9 ± 0.9 g, were randomly divided into groups of 7 and each group was assigned to 1 of the 9 dietary treatments. The rats were purchased from B&K Universal.

All diets contained a basal diet mixture (329 g/kg, dry weight) prepared according to Henningsson et al. (6). Wheat starch (582–590 g/kg, dry weight) was used to adjust the dry matter content. This type of starch is completely digested and absorbed and therefore does not contribute to any hindgut fermentation (14). The ICs were added at a level of 80 g/kg diet (corresponding to 89 g pectin/kg dry weight and 80 g lactitol and inulin per kg dry weight); amounts of the probiotic strains were adjusted to give each rat 1 × 10^10 cfu/d of Bb-12 (1 g/kg, dry weight) or 1 × 10^7 cfu/d of UCC500 (7 g/kg, dry weight). The diets containing the probiotics were kept refrigerated until fed to the rats.

The feed intake was restricted to 12 g dry weight/d, and water was freely available. The rats were allowed to adapt to the diet for 7 d; a 5-d experimental period followed, when feeds and feed residues were collected daily. The feeds were stored at −20°C and then freeze-dried and milled before being analyzed for remaining IC. During the following 24 h of the experiment, fresh feeds were collected on dry ice for CA determination and bacterial counts (6-d feeds). The 6-d feeds from rats fed UCC500 were frozen and sent for microbial analysis (UCC), whereas feeds from rats fed Bb-12 were collected in transport medium, consisting of 0.9% NaCl, 0.1% peptone, 0.1% Tween 80, and 0.02% cysteine and analyzed directly. At the end of the experiment, the rats were killed using carbon dioxide narcosis and the cecum and proximal and distal colon were removed. Cecal tissue weight, content weight, and pH were measured directly, and contents from the different parts of the hindgut were frozen and stored at −40°C until analysis of CAs. The Ethics Committee for Animal Studies at Lund University approved the animal experiments.

#### Analytical methods

**Carboxylic acids.** GLC was used to analyze the amount of the following carboxylic acids: formic, acetic, propionic, isobutyric, butyric, isovaleric, valeric, caproic, heptanoic, lactic, and succinic acid (6,15). The intestinal content and the fecal samples were homogenized (using a Polytron homogenizer, Kinematica) together with an internal standard (2-ethylbutyric acid, Sigma Chemical). Hydrochloric acid was added to protonate the CAs and to enable their extraction in diethyl-ether.

**Indigestible carbohydrates.** The amount of inulin and lactitol in the raw material was assumed to be as specified by the manufactures (995 and 990 g/kg dry weight, respectively). Dietary fiber in pectin was isolated using the method developed by Asp et al. (16). The composition of the dietary fiber in the isolated fiber residues and in feces was analyzed using GLC for the neutral sugars as their alditol acetates, and using a spectrophotometric method for the uronic acids (17). The amount of dietary fiber in pectin was 900 g/kg dry weight. The amounts of fructans in feces were quantified using the AOAC method 999.03 (18). In this method, fructooligosaccharides are treated with fructanase (exo-inulinase) and the amount of fructose is quantified with the R-hydroxy-benzoic acid hydrate reducing-sugar method. Lactitol was extracted from feces in ethanol (50%, v:v) for 30 min at room temperature according to Ekvall et al. (19). Lactitol was then analyzed using high performance anion exchange chromatography with pulsed amperometric detection ( Dionex 500). NaOH (15–200 mmol) was used as the eluent at a flow rate of 1.0 mL/min and arabinose was used as internal standard.

**Microbial analysis.** Bifidobacteria and lactobacilli were grown at 37°C for 3 d on Wilkins Chalgren Agar (Oxoid) with mupirocin or on LBS agar (lactobacillus-selective agar), respectively (20,21). Identification of bifidobacteria was made by genus-specific PCR and randomly amplified polymorphic DNA (RAPD) (22). Typing of lactobacilli was performed by RAPD (23) on colonies that were picked randomly from medium selective for lactobacilli (21).

#### Calculations and statistical evaluation

The design of the experiment resulted in 3 control groups given the different ICs and 6 groups given the test diets, which contained one of the ICs together with either Bb-12 or UCC500. All analyses were performed at least in duplicate. The maximum error of the analyses was <5%. The cellulose pools of CAs were calculated as the levels of each acid (μmol/g) multiplied by the weight of the cecal content. The values were extrapolated to a complete intake of IC (4.8 g) and thus corrected for the small amounts of feed residues. Bulking capacity was calculated as fecal dry weight in g/IC eaten. The body weight gain was calculated in g/IC eaten, and the cecal content was calculated in g/IC eaten. All statistical evaluations were performed with the Minitab statistical software (Release 13.32). Data were tested using 2-way ANOVA to determine the effects of indigestible carbohydrates, probiotics, and their interactions, (*P* < 0.05). Then, the probiotic effects were evaluated by comparing data from rats given diets containing Bb-12 or UCC500 with data from rats fed the corresponding IC diet. One-way ANOVA was then used followed by Dunnett’s procedure (*P* < 0.05). When results from pectin, inulin, and lactitol, without any probiotics were compared, 1-way ANOVA was used followed by Tukey’s procedure (*P* < 0.01).
Correlations between acid concentrations in the distal colon and feces were calculated using linear regression ($P < 0.05$).

**Results**

**Weight gain, feed intake, cecal pH, and fecal weight**
The body weight gain was similar for all rats, except for the rats given inulin and UCC500, which gained less weight than those fed only inulin ($P < 0.01$) (Table 1). The bulking capacity decreased ($P < 0.05$) when rats were fed lactitol and Bb-12, whereas it increased when the rats were fed this strain with pectin ($P < 0.01$). The weight of the cecal content increased in rats when the probiotic strains were added to lactitol ($P < 0.05$), whereas it decreased when UCC500 was added to the pectin diet ($P < 0.05$). The probiotic bacteria had no effect on the cecal pH. Somewhat higher amounts of the ingested pectin were excreted in feces of rats when UCC500 was included in the diet ($P < 0.05$) than without this strain, whereas no such effects were seen in combination with Bb-12 or in any other pre- and probiotic combination. In rats fed inulin, 5% was excreted independently of probiotics, and no lactitol was excreted by any of the rats.

**Microbial analyses**
Bb-12 survived the passage through the gut and was a part of the microflora in feces, but none of the colonies isolated from rat feces had the same RAPD PCR profile as UCC500.

**CAs in the hindgut of rats fed pectin, inulin, and lactitol**
The IC were all readily fermented (Table 1). However, rats fed pectin had larger cecal pools ($P < 0.001$) and concentrations ($P < 0.01$) of CAs than those fed inulin and lactitol (Table 2). These 2 substrates instead exhibited higher concentrations of CAs than pectin in the distal colon of rats ($P < 0.001$), indicating a slower fermentation of these materials. In the cecum of rats, the proportion of propionic acid was comparatively higher with inulin and lactitol in the diet than with pectin ($P < 0.001$). Further, in the distal part of colon, in rats fed inulin, high proportions of propionic acid ($P < 0.001$) and butyric acid ($P < 0.001$) were formed compared with those fed pectin and lactitol. The proportion of lactic acid increased along the hindgut of rats except for inulin.

**Effects of Bb-12 and UCC500 on the formation of CAs in the hindgut of rats**

**Bb-12.** The cecal pool of CAs was larger ($P < 0.01$) when rats were given inulin and Bb-12 than inulin without this probiotic strain (Table 2). There were no effects on the cecal pool of CAs with this strain when combined with pectin and lactitol.

The concentration of CAs was larger throughout the hindgut of rats fed pectin with Bb-12 ($P < 0.05$-$P < 0.001$) compared with pectin alone (Table 2). The increase of CAs was most pronounced in the distal part of colon, where it was more than doubled ($P < 0.001$). The increase could be ascribed to acetic acid and in the distal part, also to propionic and butyric acid.

When rats were fed inulin and Bb-12, the cecal concentration of CAs was larger than in those fed inulin only ($P < 0.05$), whereas the concentration was lower in the distal part of colon ($P < 0.01$). The higher amount of CAs in the cecum of rats was due mainly to propionic acid, whereas the lower concentration in the distal part of the colon of rats was due to all CAs except lactic acid resulting in an increased proportion of lactic acid ($P < 0.001$).

Bb-12 had no effects on the cecal concentration of CAs in rats fed lactitol. However, in the distal part of colon, the concentration of total CAs was lower when the rats were fed lactitol and Bb-12 than lactitol without this strain ($P < 0.001$). In the cecum, the proportions of different CAs were changed, with a lower proportion of propionic acid when rats were fed lactitol and Bb-12 ($P < 0.01$) compared with lactitol only, whereas the proportion of lactic acid was higher ($P < 0.001$).

**UCC500.** When the different diets were supplemented with UCC500, the cecal pool of CAs decreased in rats fed pectin ($P < 0.05$), whereas it increased in rats fed lactitol ($P < 0.001$). Rats fed inulin were not affected.

In rats fed pectin and UCC500, the fermentation was shifted in part from the cecum to the distal part of colon, as judged from the lower cecal pools ($P < 0.05$) and higher distal concentrations of CAs ($P < 0.01$) compared with rats fed pectin only. The molar proportions were only slightly affected; an exception was the significantly lower proportion of lactic acid in the distal part of colon ($P < 0.001$). The concentration of CAs in rats fed UCC500 and inulin or lactitol were not affected. However, there was a lower proportion of acetic acid in the distal part of the

| TABLE 1 | Body weight gain, bulking capacity, cecal content, and cecal pH in rats fed diets containing pectin, inulin, or lactitol supplemented with Lactobacillus salivarius (UCC500) or Bifidobacterium lactis (Bb-12)
<table>
<thead>
<tr>
<th></th>
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<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Diet</strong></td>
<td><strong>Body weight gain, g/g feed</strong></td>
<td><strong>Bulking capacity, g feces/g IC</strong></td>
<td><strong>Cecal content, g/g IC</strong></td>
<td><strong>Cecal pH</strong></td>
<td><strong>Fecal excretion of IC, %</strong></td>
</tr>
<tr>
<td>Pectin</td>
<td>0.20 ± 0.04</td>
<td>0.51 ± 0.02</td>
<td>0.41 ± 0.03</td>
<td>6.5 ± 0.1</td>
<td>4 ± 0</td>
</tr>
<tr>
<td>+UCC500</td>
<td>0.20 ± 0.02</td>
<td>0.62 ± 0.05</td>
<td>0.28 ± 0.02*</td>
<td>6.5 ± 0.1</td>
<td>12 ± 1*</td>
</tr>
<tr>
<td>+Bb-12</td>
<td>0.25 ± 0.00</td>
<td>0.70 ± 0.05**</td>
<td>0.39 ± 0.04</td>
<td>6.5 ± 0.1</td>
<td>7 ± 0</td>
</tr>
<tr>
<td>Inulin</td>
<td>0.26 ± 0.02</td>
<td>0.65 ± 0.02</td>
<td>0.31 ± 0.03</td>
<td>6.9 ± 0.1</td>
<td>5 ± 0</td>
</tr>
<tr>
<td>+UCC500</td>
<td>0.15 ± 0.02**</td>
<td>0.68 ± 0.02</td>
<td>0.30 ± 0.01</td>
<td>6.9 ± 0.1</td>
<td>5 ± 0</td>
</tr>
<tr>
<td>+Bb-12</td>
<td>0.22 ± 0.03</td>
<td>0.68 ± 0.02</td>
<td>0.31 ± 0.02</td>
<td>6.9 ± 0.0</td>
<td>5 ± 0</td>
</tr>
<tr>
<td>Lactitol</td>
<td>0.24 ± 0.02</td>
<td>0.45 ± 0.04</td>
<td>0.37 ± 0.06</td>
<td>6.6 ± 0.3</td>
<td>0 ± 0</td>
</tr>
<tr>
<td>+UCC500</td>
<td>0.20 ± 0.02</td>
<td>0.41 ± 0.06</td>
<td>0.61 ± 0.07*</td>
<td>6.8 ± 0.1</td>
<td>0 ± 0</td>
</tr>
<tr>
<td>+Bb-12</td>
<td>0.20 ± 0.02</td>
<td>0.30 ± 0.04*</td>
<td>0.61 ± 0.06*</td>
<td>6.6 ± 0.3</td>
<td>0 ± 0</td>
</tr>
<tr>
<td>IC</td>
<td>NS</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>0.015</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Pro*</td>
<td>0.018</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>IC×Pro</td>
<td>NS</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>NS</td>
<td>0.029</td>
</tr>
</tbody>
</table>

1 Values are means ± SEM, $n = 7$. *Different from rats fed diets without bacteria: $*P < 0.05$, $**P < 0.01$, $***P < 0.001$.
2 Probiotics.
TABLE 2  
Carboxylic acids in the hindgut of rats fed diets containing pectin, inulin, and lactitol supplemented with Lactobacillus salivarius and Bifidobacterium lactis UCC500 and Bb-12.1,2

<table>
<thead>
<tr>
<th>Cecum</th>
<th>Pectin3</th>
<th>UCC500</th>
<th>Bb-12</th>
<th>Inulin4</th>
<th>UCC500</th>
<th>Bb-12</th>
<th>Lactitol5</th>
<th>UCC500</th>
<th>Bb-12</th>
<th>IC</th>
<th>F</th>
<th>Pro</th>
<th>F×Pro</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetic</td>
<td>55 ± 1</td>
<td>60 ± 2</td>
<td>64 ± 1***</td>
<td>52 ± 1</td>
<td>50 ± 1</td>
<td>49 ± 1</td>
<td>56 ± 2</td>
<td>52 ± 1*</td>
<td>55 ± 1</td>
<td>&lt;0.001</td>
<td>40 NS</td>
<td>&lt;0.001</td>
<td>7</td>
</tr>
<tr>
<td>Propionic</td>
<td>15 ± 1*</td>
<td>16 ± 1</td>
<td>14 ± 1</td>
<td>23 ± 1</td>
<td>24 ± 1</td>
<td>31 ± 1***</td>
<td>24 ± 20</td>
<td>18 ± 1***</td>
<td>19 ± 1**</td>
<td>&lt;0.001</td>
<td>94 0.007</td>
<td>5 &lt;0.001</td>
<td>15</td>
</tr>
<tr>
<td>Butyric</td>
<td>6 ± 1</td>
<td>10 ± 1</td>
<td>11 ± 1</td>
<td>14 ± 1</td>
<td>12 ± 1</td>
<td>12 ± 1</td>
<td>10 ± 04</td>
<td>12 ± 1</td>
<td>9 ± 1</td>
<td>0.012</td>
<td>5 NS</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>Lactic</td>
<td>5 ± 06</td>
<td>5 ± 1</td>
<td>5 ± 1</td>
<td>2 ± 00</td>
<td>4 ± 00</td>
<td>2 ± 0</td>
<td>2 ± 04</td>
<td>6 ± 0***</td>
<td>6 ± 0***</td>
<td>&lt;0.001</td>
<td>14 &lt;0.001</td>
<td>15 &lt;0.001</td>
<td>7</td>
</tr>
<tr>
<td>Succinic</td>
<td>8 ± 1</td>
<td>5 ± 1*</td>
<td>3 ± 1***</td>
<td>4 ± 1</td>
<td>6 ± 1</td>
<td>2 ± 1</td>
<td>4 ± 1</td>
<td>8 ± 0**</td>
<td>8 ± 1*</td>
<td>&lt;0.001</td>
<td>8 &lt;0.001</td>
<td>7 &lt;0.001</td>
<td>9</td>
</tr>
<tr>
<td>Minor3</td>
<td>6 ± 1</td>
<td>4 ± 06</td>
<td>3 ± 0***</td>
<td>5 ± 0</td>
<td>4 ± 0</td>
<td>4 ± 0</td>
<td>4 ± 0</td>
<td>3 ± 1</td>
<td>NS</td>
<td>0.01</td>
<td>5 NS</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total6</td>
<td>835 ± 8.07</td>
<td>776 ± 5.8</td>
<td>1153 ± 8.0</td>
<td>596 ± 2.3</td>
<td>616 ± 1.2</td>
<td>70.0 ± 3.4</td>
<td>525.2 ± 2.4</td>
<td>625.4 ± 1.1</td>
<td>625 ± 6.2</td>
<td>&lt;0.001</td>
<td>35 &lt;0.001</td>
<td>10 0.005</td>
<td>4</td>
</tr>
</tbody>
</table>

μmol/g

| Proximal colon | Acetic | 56 ± 2 | 52 ± 2 | 60 ± 2 | 51 ± 2 | 50 ± 2 | 59 ± 1*** | 51 ± 2 | 56 ± 1* | 60 ± 1*** NS | <0.001 | 16 NS |
| Propionic | 10 ± 1 | 7 ± 1* | 9 ± 1 | 15 ± 2 | 23 ± 1*** | 19 ± 1 | 14 ± 2 | 9 ± 1 | 11 NS | <0.001 | 54 NS | <0.001 | 7 |
| Butyric | 6 ± 1 | 4 ± 1 | 5 ± 0 | 14 ± 2 | 12 ± 2 | 6 ± 1*** | 8 ± 1ab | 5 ± 1** | 5 ± 1** | <0.001 | 19 0.001 | 8 0.005 | 4 |
| Lactic | 22 ± 2 | 27 ± 3 | 17 ± 3 | 14 ± 3 | 9 ± 1* | 11 ± 1 | 20 ± 3 | 22 ± 2 | 14 ± 1 | <0.001 | 22 0.008 | 5 0.026 | 3 |
| Succinic | 1 ± 0 | 5 ± 3** | 4 ± 1 | 3 ± 1 | 1 ± 1 | 1 ± 0 | 4 ± 1 | 4 ± 1 | 5 ± 1 | <0.001 | 11 NS | 0.008 | 4 |
| Minor3 | 5 ± 1 | 5 ± 1 | 5 ± 0 | 3 ± 0 | 5 ± 0 | 4 ± 0 | 3 ± 0 | 4 ± 0 | 5 ± 0* NS | NS | NS |
| Total6 | 483 ± 5.2 | 612 ± 4.4 | 882 ± 4.4*** | 646.8 ± 1.8 | 70.0 ± 2.7 | 582 ± 1.3 | 657.3 | 696 ± 2.7 | 481 ± 2.4 | 0.030 | 4 NS | <0.001 | 20 |

μmol/g

| Distal colon | Acetic | 48 ± 2b | 53 ± 1 | 55 ± 1** | 46 ± 34 | 34 ± 2*** | 44 ± 2 | 56 ± 2b | 54 ± 1 | 56 ± 1 | <0.001 | 60 0.004 | 6 <0.001 | 7 |
| Propionic | 9 ± 1a | 13 ± 2 | 15 ± 1 | 23 ± 2 | 25 ± 1 | 14 ± 3** | 14 ± 1a | 12 ± 1 | 12 ± 1 | <0.001 | 28 0.048 | 3 <0.001 | 8 |
| Butyric | 6 ± 1 | 8 ± 1 | 11 ± 1** | 19 ± 2 | 21 ± 2 | 7 ± 2** | 5 ± 0 | 8 ± 1* | 6 ± 1 | <0.001 | 41 0.002 | 7 <0.001 | 15 |
| Lactic | 28 ± 4a | 12 ± 3** | 9 ± 1*** | 6 ± 3 | 13 ± 2 | 25 ± 5*** | 16 ± 2a | 17 ± 2 | 16 ± 1 | NS | NS | <0.001 | 12 |
| Succinic | 4 ± 0 | 9 ± 1* | 5 ± 1 | 2 ± 2 | 3 ± 1 | 1 ± 0 | 6 ± 1 | 6 ± 1 | 6 ± 0 | <0.001 | 16 0.017 | 4 NS |
| Minor3 | 5 ± 0 | 5 ± 1 | 5 ± 0 | 4 ± 2b | 4 ± 2 | 9 ± 2** | 3 ± 0 | 3 ± 1 | 4 ± 0 | 0.003 | 6 0.001 | 7 <0.001 | 6 |
| Total6 | 49.3 ± 3.2 | 75.3 | 53.3** | 122.0 | 71.1*** | 884.6 ± 6 | 63.9 | 78.5 | 2.9 | 63.2 | 6.0** | 86.3 | 5.18 | 72.8 | 3.0 | 57.2 | 3.3*** NS | <0.001 | 36 |

1 Values are means ± SEM, n = 7 or percentage of total CA (%). Asterisks indicate different from rats fed diets without bacteria: *P < 0.05, **P < 0.01, ***P < 0.001.
2 Means between pectin, inulin, and lactitol, i.e., those without any added probiotics, without a common letter differ, P < 0.01.
3 Formic, isobutyric, valeric, isovaleric, caproic, and heptanoic acid.
4 Formic, acetic, propionic, butyric, isobutyric, valeric, isovaleric, caproic, heptanoic, succinic, and lactic acid.

Discussion

The increasing evidence that propionic and butyric acid have positive effects on colonic health has resulted in an interest in modulating the formation of CAs through diet. In this study in rats, 3 fermentable carbohydrates were combined with 2 probiotic strains, to determine the possibility of increasing the amount of CAs formed, as well as to study whether the CA profile and the site of release of CAs could be changed. These carbohydrates were chosen because of their capacity to form different amounts and types of CAs, and their different rates of fermentation (4–6). Supplementation with probiotics was shown to further affect the total formation of CAs, the proportions of different CAs and the site of release. However, different effects were seen with the various combinations of probiotics and fermentable carbohydrates. The rats were given the diets for 13 d, which was shown to be sufficient for optimal fermentation of dietary fiber components and a quite stable pattern of CAs (24,25).

The type of inulin used in this study has a low solubility; thus, it was fermented comparatively slowly, giving lower amounts of CAs in the cecum than other fructooligosaccharides (5). However, when Bb-12 was added to the diet, the cecal concentration of CAs increased, whereas it decreased in the distal part of colon. Thus, Bb-12 seems to stimulate the cecal fermentation of inulin, resulting in lower amounts of substrate reaching the distal part of the colon. This was also verified by the similar amounts of fructans excreted in feces showing a similar total fermentability. The increased concentration of CAs in the cecum was the result of a specific increase in propionic acid. Interestingly, the proportion reached a level that was as high as that for inulin with high solubility, a fructooligosaccharide that was previously shown to be especially prone to generating propionic acid (5). In the distal part of the colon, on the other hand, the proportions of propionic and butyric acid decreased, whereas that of lactic acid...
The mechanisms behind this could be that the colonic microbiota metabolizes pectin via other pathways when Bb-12 is added, as indicated by the different profiles of CAs. In the distal part of the colon, for example, the proportion of lactic acid decreased, whereas that of propionic, butyric, and acetic acid increased. Because pectin stimulates the growth of both bacterio- rodes and eubacterium (28,29), supplementation with Bb-12 leads to a more complex situation in the hindgut with more metabolic interactions among the microbial species than with inulin. The bacteria per se could also contribute to the amount of substrate available in the colon by upregulating mucus production. It was shown that probiotics increase the production of mucins from HT-29 cells (30) as well as from the jejunum and ileum of rats (Ahnré, S., unpublished results).

The low amounts of CAs in the cecum of rats fed lactitol indicate a low degree of cecal fermentation; this was also observed by others (31). However, lactitol was readily fermented during passage through the hindgut, as evidenced by the finding that no lactitol was excreted in feces. This was the case regardless with or without the addition of probiotics. In rats fed lactitol and Bb-12, the concentration of CAs in the distal part of the colon was even lower than without this strain. This could be due to an accelerated formation and absorption of CAs when this strain is included, leading to less substrate being delivered to the distal part. Similar observations were made by others (32). For example, when Lactobacillus reuteri was added to a diet containing a mixture of easily fermentable fibers (oat fiber, potato fiber, pectin, and pea fiber), there was a general decrease in the fecal concentrations of CAs in rats (32). When UCC500 was added to the diet containing lactitol, the cecal pool of CAs increased, indicating an overall increase in bacterial activity or a change in the composition of the cecal microbiota.

When UCC500 was added to the pectin diet, there was a decrease in the cecal pool of CAs in the rats, whereas the concentration of CAs in the distal colon was higher, indicating that the site of fermentation had been changed to the distal part of the colon. A reason for that could be that this strain inhibits the activity of pectin-degrading enzymes in the cecum, resulting in a reduced amount of fermentation and reduced formation of CAs. As a consequence, more substrate would be available distally, which was also indicated by the higher fecal excretion of pectin when UCC500 was added. Interestingly, there was an increase in the concentration of propionic and butyric acids in the distal part of the colon when UCC500 was added, which may be relevant because most colonic diseases such as ulcerative colitis and cancer appear primarily in the distal part of colon. In this respect, it is interesting to note that there was a good correlation between the concentrations of acetic, propionic, and butyric acid in the feces and in the distal colon because most measurements of short-chain fatty acids on humans are made primarily in feces.

An important issue is whether the probiotic bacteria survive the passage through the gastrointestinal tract. Bb-12 was detected in all fecal samples, whereas UCC500 was not. It can therefore be questioned whether UCC500 survived passage through the hindgut. However, the changes in both the amount and the profile of CAs formed in the hindgut of rats indicate that the strain remained alive through the entire colon. One reason for this could be that the colonic microbiota metabolizes pectin via other pathways when Bb-12 is added, as indicated by the different profiles of CAs. In the distal part of the colon, for example, the proportion of lactic acid decreased, whereas that of propionic, butyric, and acetic acid increased. Because pectin stimulates the growth of both bacterio-

increased. Carbohydrates fermented by bifidobacteria were reported to use a different metabolic pathway, the “bifid shunt,” producing high amounts of acetic acid and lactic acid (in the molar ratio 3:2) (26), whereas propionic and butyric acid are not produced through this pathway. We speculate whether the number of bifidobacteria in relation to the amount of fermentable substrate is of importance for the profile of CAs formed. It appears that high amounts of lactic acid are formed when the substrate is limiting, as in the distal part of the colon in rats fed Bb-12 and inulin. Propionic acid, on the other hand, seems to be formed when there is a surplus of substrate, as in the cecum of these rats. Interestingly, Bb-12 combined with inulin (Raftiline and Raftilose) was shown to raise the integrity of tight junction in an in vitro model, a factor that was suggested to protect the mucosal barrier from disruption (27).

When rats were fed pectin and Bb-12, the concentrations of CAs were larger throughout the hindgut of rats, especially in the distal part of the colon, compared with rats fed pectin only, leading to a higher net amount of CAs (P < 0.05–0.001). Because the fecal excretion of pectin was very similar with and without the presence of Bb-12 in the diet, it appears that pectin was more effective in generating CAs in the presence of Bb-12.

The mechanisms behind this could be that the colonic micro-

Correlation between acetic, propionic, and butyric acid in the distal colon and feces of rats fed diets containing pectin (A), inulin (B), and lactitol (C) supplemented with Lactobacillus salivarius (UCC500) and Bifidobacterium lactis (Bb-12).
it was delivered at a lower concentration. However, the recommended dose per day for probiotics is $1 \times 10^9$ cfu; therefore, the amount given per day ought to be sufficient (9).

We conclude that the probiotic bacteria studied here affect both the CA pattern and the site of CA release in the hindgut of rats. However, the ICs had more pronounced effects on both the profiles and the cecal concentrations and pools of CAs, as judged from the higher F-values (Table 2). In the distal part of the colon, the combination of ICs and probiotics was of great importance for the CA concentrations, as demonstrated by the high interaction. Bb-12 in combination with inulin specifically increased the cecal pool of propionic acid in the rats, whereas the amount of lactic acid increased in the distal colon, possibly due to the limited amount of substrate in relation to the number of probiotics in this part. Bb-12 decreased the concentration of CAs in the distal colon of rats fed lactitol, indicating an accelerated absorption of CAs, whereas the concentration of CAs was higher throughout the hindgut of rats fed pectin and Bb-12. When rats were fed UCC500 and pectin, there was a shift in the amount of CAs from the cecum to the distal part of the colon, whereas the formation of CAs in combination with lactitol in the cecum of rats was stimulated.

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**Literature Cited**