Oral Administration of *Butyrivibrio fibrisolvens*, a Butyrate-Producing Bacterium, Decreases the Formation of Aberrant Crypt Foci in the Colon and Rectum of Mice\(^1,2\)

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**ABSTRACT**  *Butyrivibrio fibrisolvens*, a butyrate-producing ruminal bacterium, was evaluated for use as a probiotic to prevent colorectal cancer. Oral administration to Jcl:ICR mice of a new strain of *B. fibrisolvens* (MDT-1) that produces butyrate at a high rate (10^9 cfu/dose) increased the rate of butyrate production by fecal microbes, suggesting that MDT-1 can grow in the gut. The number of colorectal aberrant crypt foci (ACF), putative neoplastic lesions induced by 1,2-dimethylhydrazine, was reduced after MDT-1 administration (10^9 cfu/dose, 3 times/wk for 4 wk). The number of aberrant crypts (ACs), number of foci having 3 or 4 ACs per focus, and the percentage of mice having 3 or 4 ACs per focus were also reduced, suggesting that the progression of lesions was suppressed by MDT-1. Interestingly, the MDT-1 cell homogenate did not have a similar beneficial effect. MDT-1 had low β-glucuronidase activity, and administration of MDT-1 reduced the β-glucuronidase activity in the colorectal contents. The numbers of natural killer (NK) and NKT cells in the spleen were markedly enhanced in response to MDT-1. Decreased β-glucuronidase activity and increased numbers of NK and NKT cells and butyrate production may explain in part why MDT-1 administration suppressed ACF formation. These results suggest that colorectal cancer may be prevented or suppressed by the utilization of MDT-1 as a probiotic. Administration of MDT-1 had no harmful effect on the health of mice at least for 3 mo. J. Nutr. 135: 2878–2883, 2005.

**KEY WORDS:** *Butyrivibrio fibrisolvens* • intestinal bacteria • probiotic • colon cancer • butyrate

SCFA, particularly butyrate, produced by intestinal microbiota play important roles in the physiology and metabolism of the intestine and intestinal mucosa (1,2). In addition to serving as a preferred energy source for colonocytes (3–5), butyrate has been implicated in protection against colon cancer and ulcerative colitis (6). The incidence of human colorectal cancer is increasing in developed countries (7–9), and large intestinal disease is also a common problem in small animal practice (10). Thus, augmentation of butyrate production in the intestine would be desirable for the maintenance of colonic health in both humans and pet animals.

Butyrate is produced by intestinal bacteria from carbohydrates such as resistant starch, dietary fiber, and fructooligosaccharides (8,9) that escape digestion in the small intestine. The effects of increasing quantities of low-digestible dietary carbohydrates on the supply of butyrate were investigated in experimental animals, including pet animals (10,11). Similar studies in rodents also investigated the ability of low-digestible carbohydrates to protect against experimentally induced tumors (8,9,12). The results of these studies suggest that a stable butyrate-producing colonic ecosystem resulting from resistant starch and fiber in the diet can reduce the incidence of colonic diseases (9,13).

Among butyrate-producing bacteria, *Clostridium butyricum* has been used in humans and animals of far-east Asia to ameliorate diarrhea and constipation (14,15). Recently, administration of this bacterium was shown to suppress chemically induced aberrant crypt foci (ACF), putative neoplastic lesions, in the rat colon (16) and experimental colitis in rats (17,18). Increased production of butyrate was suggested to be one of the reasons (17,18) for the beneficial effects of *C. butyricum*.

We are interested in the utilization of *Butyrivibrio fibrisolvens*, a representative butyrate-producing ruminal bacterium with a high capacity to produce butyrate (19), as a probiotic. The ability of *B. fibrisolvens* to produce conjugated linoleic acid (CLA) (20) may also benefit health (21) by prevention of carcinogenesis (22), atherosclerosis (23), and tumorogenesis (24,25), and improvement of hyperinsulinemia (26) and immune functions (27,28).

*B. fibrisolvens* also resides in the intestine of humans (29),

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\(^2\) Supplemental Tables 1 and 2 are available as Online Supporting Material with the online posting of this paper at www.nutrition.org.

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Because addition of \(B. \text{fibrisolvens}\) to a culture of mixed fecal microbes of dogs resulted in increased butyrate production (30), introduction of \(B. \text{fibrisolvens}\) as a probiotic, or stimulation of its growth, may increase butyrate and CLA production in the gut. We hypothesize that administration of probiotic \(B. \text{fibrisolvens}\) to humans will also increase butyrate and CLA production in the gut.

### MATERIALS AND METHODS

#### Bacterial strains and culture conditions.

\(B. \text{fibrisolvens}\) strains (ATCC19171, ATCC51255, OB156, A38, and TH1) were obtained as described previously (31). Another strain of \(B. \text{fibrisolvens}\), called MDT-1, was newly isolated from the rumen of a Japanese native goat by the roll tube method (32), and identified according to Bryant (33). \(C. \text{butyricum}\) MYAIRI 588 was provided by Meiji Seika Kaisha.

All bacterial strains were grown in a growth medium for \(B. \text{fibrisolvens}\), which consisted of a basal medium (31) and mixtures of SCFA, vitamins, and trace minerals (34). Details of culture conditions and procedures were as described previously (31). Cell growth was estimated by optical density at 600 nm (OD_{600}). Cells were harvested at OD_{600} 1.3–1.5 (late-log growth), and immediately cooled on ice. Viable cells were counted using Hugl's roll tube method in a medium containing 2% agar (32). Five tubes were measured for each sample, and colony numbers were counted.

#### Mice.

Male Crl:ICR mice (4 wk old; CLEA Japan) were used in all experiments. Mice were housed in plastic cages with wire tops, and permitted free access to a commercial diet (CE-2, CLEA Japan) and water. The diet contained 249 g crude protein, 46 g crude fat, 31 g crude fiber, 67 g ash, 514 g nitrogen-free extracts, and 14.51 MJ/kg. General animal handling and care procedures were carried out according to the "Guideline for the Care and Use of Laboratory Animals, Meiji University."

#### Oral administration of bacteria to mice.

\(B. \text{fibrisolvens}\) MDT-1 and \(C. \text{butyricum}\) grown to late-log stage were collected by centrifugation (5000 \(g\), 10 min, 4°C) and washed with 0.8% NaCl. Washed cells, resuspended in 0.8% NaCl (10^{15} cfu/L), were used directly as intact cells, or were disrupted by sonication (20 kHz, 60 W, 50% duty cycle) until >95% were broken as estimated by microscopy. Unbroken cells were removed by centrifugation (5000 \(g\), 10 min, 4°C) and the supernatant was used as a cell homogenate. Intact cells suspensions and cell homogenates were administered to mice using a stomach tube at a level of 0.1 mL or 10^{5} cfu/ (mouse \cdot dose) [or (mouse \cdot d)].

#### Expt. 1 (butyrate production in the intestine).

\(B. \text{fibrisolvens}\) MDT-1 or \(C. \text{butyricum}\) live cells (10^{6} cfu/dose), or a 0.8% NaCl control solution was administered to mice using a stomach tube (5/group). An aliquot of freshly excreted feces was immediately inoculated to a growth medium consisted of the basal medium described above and clarified ruminal fluid [3:1, 35]. Cultures were grown anaerobically at 37°C for 3 h, and samples for the determination of fermentation products were collected at 1-h intervals to estimate the initial rate of organic acid production. Organic acids were quantified by HPLC as described previously (36).

#### Expt. 2 (suppression of ACF).

The protocol of this experiment is outlined in Figure 1. The 6 groups were administered the following treatments: Group A, control (saline); Group B, 1,2-dimethylhydrazine (DMH); Group C, DMH and \(B. \text{fibrisolvens}\) MDT-1 1 time/wk; Group D, DMH and MDT-1 3 times/wk; Group E, DMH and cell homogenates of MDT-1 3 times/wk; and Group F, DMH and \(C. \text{butyricum}\) 3 times/wk. Oral administration of bacterial preparations was initiated and continued intermittently for 4 wk. ACF were induced using DMH as described by Tudek et al. (37). Briefly, DMH [Aldrich; 0.75 mg/(mouse \cdot d)] was administered to mice using a stomach tube both 6 and 10 d after the first administration of bacterial preparations. After 4 wk, mice were killed by cervical dislocation under ether anesthesia and autopsied. Colonic and cecal contents were immediately cooled on ice, and then stored at −80°C until the analysis of \(\beta\)-glucuronidase activity.

#### Expt. 3 (numbers of lymphocytes in the spleen).

Intact MDT-1 cells (10^{6} cfu/dose) were administered to 5 mice (1 dose), and the mice were killed after 7 d. Spleens were harvested into 50 mmol/L potassium phosphate buffer (pH 7.0; PBS) containing 0.1% bovine serum albumin (BSA) [PBS-BSA], and squeezed to obtain lymphocytes. Erythrocytes were eliminated by immersion in lysis buffer (14 mmol/L NH_4Cl, 17 mmol/L Tris, pH 7.6) for 5 min, followed by washing once with PBS. Each pellet was resuspended in PBS-BSA, filtered through a sterile 70-μm nylon cell strainer to remove debris, and finally washed with PBS-BSA. Live mononuclear cells were enumerated by trypan blue dye exclusion, and the samples were suspended again in PBS-BSA (1.0 × 10^{10} cells/L).

The numbers of specific leukocyte subtypes were measured by immunofluorescent antibody staining using a flow cytometer (FACSCalibur, Becton Dickinson). Lymphocyte subpopulations were identified and gated using forward vs. side scatter characteristics. T cells were identified using fluorescein isothiocyanate (FITC)-conjugated rat anti-mouse CD3 (clone KT3). B cells and natural killer (NK) cells were identified using phycoerythrin (PE)-conjugated rat anti-mouse B19 (6D5) and PE-conjugated rat anti-mouse NK1.1 (PK136), respectively. All monoclonals were obtained from Beckman Coulter. Cell suspensions were incubated with antibody for 20 min at room temperature, washed with PBS, and read on the FACSCalibur; 5000–5000 events were acquired from each preparation. Control samples matched for each fluorochrome and each antibody isotype were used to set negative staining criteria. Data were analyzed using CELLQuest software (Becton Dickinson).

#### Assay of \(\beta\)-glucuronidase activity.

Bacterial cultures were centrifuged (5000 \(x\) g, 10 min, 4°C), and the supernatant was assayed to measure extracellular \(\beta\)-glucuronidase activity. The cells resuspended in 50 μL of 0.1 mol/L sodium acetate buffer (pH 5.0) containing 20% (wt/v) BSA were disrupted by sonication (<4°C), and the supernatant (cell homogenate) was assayed for intracellular \(\beta\)-glucuronidase activity. Enzyme activity was expressed as the reaction rate per milligram of cellular nitrogen (cell-N). Cell-N was determined with washed cell pellets by the Kjeldahl method (39).

The cecal and colon contents of mice were suspended in 3 volumes of sodium acetate buffer and then sonicated (<4°C). The suspensions were centrifuged (5000 \(x\) g, 10 min, 4°C), and the supernatant was used to assay \(\beta\)-glucuronidase activity. The activity of \(\beta\)-glucuronidase was determined by a modified method described for a \(\beta\)-glucuronidase assay in the bile of miniature pigs (40). Briefly, samples were incubated at 37°C for 1 h in the presence of 0.03 mol/L phenolphthalein-glucuronic acid (Sigma-Aldrich), and then the reaction product, phenolphthalein, was determined using the protocol described for a \(\beta\)-glucuronidase assay in the intestine.

### Figure 1

Protocol for the animal expt. 2. Group A: administration of 0.8% NaCl solution (N; control), Group B: administration of DMH (D), Group C: administration of DMH and intact \(B. \text{fibrisolvens}\) MDT-1 (B) 1 times/wk; Group D: administration of DMH and intact MDT-1 3 times/wk, Group E: administration of DMH and MDT-1 cell homogenates (Bh) 3 times/wk, Group F: administration of DMH and intact \(C. \text{butyricum}\) (C) 3 times/wk, A: Autopsy.

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terminated by measuring the absorbance at 550 nm with a multilabel counter (ARVO SX 1420, Wallac).

Statistical analysis. In Expt. 1, data were analyzed by 2-way ANOVA (bacterial administration and time after administration). Tukey's test was done when the interaction was significant. In Expts. 2 and 3, data were analyzed by 1-way ANOVA, and Tukey's test was used when the F-test was significant. Differences with P-values < 0.05 were considered significant. Data are expressed as means ± SEM. All statistical analyses were performed with the SigmaStat Statistical Analysis System (Jandel Scientific).

RESULTS

Butyrate-producing ability of B. fibrisolvens MDT-1. A new strain of B. fibrisolvens, MDT-1, produced a higher proportion of butyrate than other strains of B. fibrisolvens and C. butyricum, P < 0.05 (Supplemental Table 1). The growth rate of MDT-1 did not differ from the rates of B. fibrisolvens TH1 and C. butyricum MIYARI 588, and was higher than those of the other strains of B. fibrisolvens (P < 0.05). Thus, MDT-1 produced butyrate at a higher rate than other strains examined.

Activity of β-glucuronidase in B. fibrisolvens. β-Glucuronidase activity was not detected in the cell homogenate of 6 strains of B. fibrisolvens, whereas the activity in the culture supernatant was 1.5–3.1 nmol/(h · mg cell-N), indicating that B. fibrisolvens secretes β-glucuronidase (Supplemental Table 2). Similar results were obtained with C. butyricum MIYARI 588. β-Glucuronidase activities in C. perfringens 1290 and E. coli K12 were ~9-fold higher than from B. fibrisolvens. Furthermore, these 2 bacteria demonstrated cell-associated β-glucuronidase activities that were 430- and 150-fold higher, respectively, than levels of the secreted enzyme. Thus, β-glucuronidase activity in B. fibrisolvens appeared to be generally low, and MDT-1 had the lowest activity.

Effect of MDT-1 on SCFA production in the intestine (Expt. 1). Administration of MDT-1 to mice resulted in a gradual increase in the initial rate of butyrate production by fecal microbes during the first 7 d (Table 1). Assuming that fecal microbes represent intestinal microbiota, and that the initial rate of butyrate production reflects the initial numbers of butyrate-producing bacteria, this increase indicates that MDT-1 administration resulted in more butyrate-producing bacteria in the gut than in the controls. The elevated rate of butyrate production was maintained until d 14, and then decreased slowly to the basal value by d 21. In contrast, when C. butyricum was administered, the initial rate of butyrate production was significantly increased on the next day, but returned to the basal level by d 3 (Table 1).

Administration of MDT-1 increased the rate of propionate production and decreased the rate of succinate production (Table 1). However, C. butyricum did not affect the production of these organic acids.

Effect of MDT-1 on ACF formation (Expt. 2). No ACF were observed in mice that were not treated with DMH. After administration of intact MDT-1 cells 1 and 3 times/wk, the

### Table 1

<table>
<thead>
<tr>
<th>Bacterium administered (1 × 10^9 cfu/mouse)</th>
<th>Succinate</th>
<th>Lactate</th>
<th>Acetate</th>
<th>Propionate</th>
<th>Butyrate</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Succinate (mmol/L · h)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>0.45 ± 0.06</td>
<td>9.74 ± 0.69</td>
<td>1.58 ± 0.33</td>
<td>0.56 ± 0.17</td>
<td>0.17 ± 0.01</td>
<td>12.50 ± 0.60</td>
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<td>0.53 ± 0.13</td>
<td>0.18 ± 0.02</td>
<td>12.15 ± 0.50</td>
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<td>3</td>
<td>0.45 ± 0.05</td>
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<td>1.60 ± 0.28</td>
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<td>0.17 ± 0.02</td>
<td>12.36 ± 0.99</td>
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<td>0.46 ± 0.07</td>
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<td>0.16 ± 0.02</td>
<td>11.45 ± 1.03</td>
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<td>7</td>
<td>0.49 ± 0.10</td>
<td>9.35 ± 0.59</td>
<td>1.73 ± 0.42</td>
<td>0.55 ± 0.11</td>
<td>0.17 ± 0.02</td>
<td>11.26 ± 1.03</td>
</tr>
<tr>
<td>14</td>
<td>0.48 ± 0.05</td>
<td>8.79 ± 1.40</td>
<td>1.46 ± 0.34</td>
<td>0.67 ± 0.12</td>
<td>0.16 ± 0.02</td>
<td>11.59 ± 1.49</td>
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<td>21</td>
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<td>9.32 ± 0.82</td>
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<td>0.64 ± 0.14</td>
<td>0.20 ± 0.02</td>
<td>12.28 ± 0.93</td>
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<tr>
<td>B. fibrisolvens Day 0</td>
<td>0.47 ± 0.09</td>
<td>9.67 ± 0.89</td>
<td>1.43 ± 0.41</td>
<td>0.63 ± 0.18</td>
<td>0.17 ± 0.02</td>
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<td>8.93 ± 1.27</td>
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<td>0.63 ± 0.21</td>
<td>0.33 ± 0.02</td>
<td>12.09 ± 0.57</td>
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<td>0.33 ± 0.11</td>
<td>7.97 ± 1.71</td>
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<td>0.73 ± 0.09</td>
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<td>1.10 ± 0.08</td>
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<td>0.23 ± 0.05</td>
<td>8.50 ± 0.93</td>
<td>1.50 ± 0.51</td>
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<td>0.18 ± 0.02</td>
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<td>C. butyricum Day 0</td>
<td>0.50 ± 0.12</td>
<td>9.30 ± 0.93</td>
<td>1.50 ± 0.34</td>
<td>0.30 ± 0.19</td>
<td>0.17 ± 0.02</td>
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<td>2.03 ± 0.41</td>
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<td>0.50 ± 0.14</td>
<td>9.17 ± 1.19</td>
<td>1.63 ± 0.23</td>
<td>0.50 ± 0.18</td>
<td>0.20 ± 0.02</td>
<td>12.00 ± 1.26</td>
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<td>5</td>
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<td>8.77 ± 1.37</td>
<td>1.67 ± 0.21</td>
<td>0.46 ± 0.19</td>
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<td>7</td>
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<td>9.37 ± 2.13</td>
<td>1.60 ± 0.18</td>
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<td>0.17 ± 0.03</td>
<td>11.94 ± 0.59</td>
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<td>1.43 ± 0.42</td>
<td>0.40 ± 0.15</td>
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<td>11.63 ± 1.64</td>
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<td>0.37 ± 0.13</td>
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<td>1.73 ± 0.31</td>
<td>0.33 ± 0.18</td>
<td>0.19 ± 0.02</td>
<td>12.02 ± 1.43</td>
</tr>
</tbody>
</table>

P-value
- **Bacterium:**
- **Day:**
- **Bacterium × Day:**

1 Values are means ± SEM, n = 5. Means in a column with superscripts without a common letter differ, P < 0.05.
2 Bacteria were administered on d 0 (1 dose).
numbers of DMH-induced ACF per mouse were reduced from 24.0 to 17.9 (75%) and 9.4 (40%), respectively (Table 2). More pronounced amelioration by intact MDT-1 was noted in the numbers of ACs per mouse, which decreased to 60% (1 time/wk) and 30% (3 times/wk) of control mice. Intact MDT-1 cells also reduced the number of foci having 3 or 4 ACs per focus per mouse. The percentage of mice having 3 or 4 ACs per focus was reduced from 70% in the control to 20% after treatment 3 times/wk (P < 0.05). Interestingly, MDT-1 cell homogenates did not reduce the incidence of ACF, suggesting that intact or viable cells are required for this effect. However, intact cells of C. butyricum had no effect on the frequency of ACF formation or the numbers of ACs per focus.

Administration of DMH decreased the body weight gain, cecum weight, colorectum weight, colorectum length, and the greater weight per length of colorectum of mice, and increased the spleen weight (data not shown, P < 0.05). Administration of intact MDT-1 cells 3 times/wk lessened these effects (P < 0.05), but neither MDT-1 cell homogenate nor intact cells of C. butyricum alleviated these effects (data not shown). Thus, live MDT-1 appears to mitigate the detrimental effects of DMH.

β-Glucuronidase activity in the cecal and colonic contents of mice treated with DMH were 50 and 140% greater, respectively, than in control mice, and provision of intact MDT-1 inhibited the increase in β-glucuronidase activity (Table 3). Neither the MDT-1 cell homogenate nor intact C. butyricum affected β-glucuronidase activities in the cecal and colonic contents.

Effect of MDT-1 on the splenic lymphocyte population (Expt. 3). Administration of intact MDT-1 cells (1 dose) did not affect the numbers of T and B cells in the spleen after 1 wk, but the numbers of NK and NKT cells (CD3/NK1.1 double positive cells) were 3.5- and 3.9-fold, respectively, greater than those of control mice (data not shown, P < 0.05).

### DISCUSSION

Administration of live MDT-1 to mice reduced the number of DMH-induced ACF (Table 2), suggesting that the presence of MDT-1 reduces the incidence of lesions (41). MDT-1 also decreased the number of ACs, the number of foci having 3 or 4 ACs per focus, and the percentage of mice having 3 or 4 ACs per focus, suggesting that the growth of lesions is suppressed by administering MDT-1 (41). However, MDT-1 cell homogenates did not reduce the incidence of ACF. It is conceivable that increased butyrate production and/or modified composition of intestinal bacteria due to live or growing MDT-1 cells alleviated the effect of DMH.

When live MDT-1 was administered, the rate of butyrate production by fecal microbes increased gradually (Table 1), suggesting that MDT-1 cell numbers increased slowly in the gut. Administration of MDT-1 increased propionate production (Table 1), suggesting that the number of bacteria producing propionate via the succinate pathway increased. Thus, the overall composition of microbial members may be altered by the growth of MDT-1.

Administration of live MDT-1 reduced β-glucuronidase activity in the contents of the colon and rectum (Table 3). Why B. fibrisolvens reduced the total β-glucuronidase activity of intestinal microbiota is not clear, but it is possible that its administration suppressed the growth of other bacteria with

### TABLE 2

Effect of the administration of B. fibrisolvens MDT-1 on the number of ACF in mice (Expt. 2)

<table>
<thead>
<tr>
<th>Administration of</th>
<th>Number of ACF¹</th>
<th>Number of ACs¹</th>
<th>1 or 2 ACs/focus¹</th>
<th>3 or 4 ACs/focus¹</th>
<th>Mice with 3 or 4 ACs/focus²</th>
<th>%</th>
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<tbody>
<tr>
<td>DMH</td>
<td>Bacterium</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>No</td>
<td>24.0 ± 4.2ᵃ</td>
<td>30.9 ± 4.3ᵃ</td>
<td>22.7 ± 4.0ᵃ</td>
<td>1.3 ± 0.1ᵃ</td>
<td>70ᵃ</td>
</tr>
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<td>Yes</td>
<td>B. fibrisolvens (intact cells), 1 time/wk</td>
<td>17.9 ± 3.0ᵇ</td>
<td>21.9 ± 2.8ᵇ</td>
<td>17.4 ± 2.8ᵇ</td>
<td>0.5 ± 0.2ᵇ</td>
<td>50ᵃᵇ</td>
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<tr>
<td>Yes</td>
<td>B. fibrisolvens (intact cells), 3 times/wk</td>
<td>9.4 ± 1.3ᶜ</td>
<td>11.5 ± 1.7ᶜ</td>
<td>9.0 ± 1.3ᵇ</td>
<td>0.4 ± 0.2ᵇ</td>
<td>20ᵇ</td>
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<tr>
<td>Yes</td>
<td>B. fibrisolvens (cell homogenate), 3 times/wk</td>
<td>19.7 ± 2.7ᵃᵇ</td>
<td>25.0 ± 3.1ᵃᵇ</td>
<td>18.7 ± 2.1ᵃ</td>
<td>0.9 ± 0.3ᵃᵇ</td>
<td>70ᵃ</td>
</tr>
<tr>
<td>Yes</td>
<td>C. butyricum (intact cells), 3 times/wk</td>
<td>25.6 ± 4.8ᵃ</td>
<td>32.6 ± 4.4ᵃ</td>
<td>24.2 ± 3.8ᵃ</td>
<td>1.4 ± 0.2ᵃ</td>
<td>70ᵃ</td>
</tr>
</tbody>
</table>

¹ Values (n/mouse) are means ± SEM, n = 10. Values in a column without a common letter differ, P < 0.05.

² Percentage of total mice having ACF. Data were evaluated at P < 0.05 by Fisher’s Exact Test.

### TABLE 3

Effect of the administration of B. fibrisolvens MDT-1 on β-glucuronidase activity in the cecal and colonic contents of mice (Expt. 2)¹

<table>
<thead>
<tr>
<th>Administration of DMH</th>
<th>Bacterium</th>
<th>Cecal content</th>
<th>Colonic content</th>
</tr>
</thead>
<tbody>
<tr>
<td>No</td>
<td>No</td>
<td>22.6 ± 4.4ᶜ</td>
<td>10.9 ± 1.4ᶜ</td>
</tr>
<tr>
<td>Yes</td>
<td>B. fibrisolvens (intact cells), 1 time/wk</td>
<td>34.1 ± 3.1ᵃᵇ</td>
<td>26.5 ± 5.7ᵃᵇ</td>
</tr>
<tr>
<td>Yes</td>
<td>B. fibrisolvens (intact cells), 3 times/wk</td>
<td>29.6 ± 3.2ᵃᶜ</td>
<td>24.3 ± 1.9ᵇ</td>
</tr>
<tr>
<td>Yes</td>
<td>B. fibrisolvens (cell homogenate), 3 times/wk</td>
<td>26.7 ± 4.1ᶜ</td>
<td>15.7 ± 3.4ᵃᶜ</td>
</tr>
<tr>
<td>Yes</td>
<td>C. butyricum (intact cells), 3 times/wk</td>
<td>36.2 ± 3.2ᵃ</td>
<td>29.3 ± 3.0ᵃ</td>
</tr>
<tr>
<td>No</td>
<td>No</td>
<td>39.6 ± 2.0ᵃ</td>
<td>30.4 ± 2.0ᵃ</td>
</tr>
</tbody>
</table>

¹ Values are means ± SEM, n = 10. Means in a column without a common letter differ, P < 0.05.
high β-glucuronidase activity, because *B. frigorolvens* produces bacteriocins (42).

DMH is a colon carcinogen that conjugates with glucuronic acid in the liver and is excreted into the intestine via the bile (43). Because the glucuronic conjugate is hydrolyzed by β-glucuronidase in the intestine, resulting in reversion to carcinogenic DMH (44), β-glucuronidase plays an important role in the induction of ACF. It was reported that a β-glucuronidase inhibitor suppressed azoxymethane (AOM)-induced carcinogenesis in the rat colon (45), and that there was a positive correlation between fecal β-glucuronidase activity and the number of AOM-induced ACF in the rat colon (46). These findings suggest that the formation of ACF is suppressed by a decrease in β-glucuronidase activity in the colon. The reduction in β-glucuronidase activity in the colorectal contents in response to MDT-1 administration appears to be one of the reasons why ACF formation was suppressed.

Generally, many genotoxic and cytotoxic compounds, ingested mainly as food, are detoxified by glucuronicidation in the liver before entering the intestine via bile. Because of its wide substrate specificity, bacterial β-glucuronidase has the ability to hydrolyze many glucuronides and may thus liberate carcinogenic aglycones in the intestinal lumen (47). Therefore, the reduction of β-glucuronidase activity by the administration of *B. frigorolvens* is of general benefit.

Great increases in the numbers of NK and NKT cells were counted in the spleen of mice administered live MDT-1. NK cells constitute a separate lineage of lymphocytes capable of being cytolytic against transformed malignant cells (48, 49). Roles of NK cells in tumor rejection and cytolytic activity against established (52). In light of these findings, increases in the number of NK cells in tumor rejection and cytolytic activity against established (52). In light of these findings, increases in the numbers of NK and NKT cells after MDT-1 administration may have an effect on the suppression of ACF formation. Moreover, this result suggests that MDT-1 exerts effects on the immune system overall, in which case MDT-1 administration may suppress carcinogenesis in organs and tissues other than the large intestine.

*C. butyricum* MIYARI 588 had no effect on ACF formation (Table 2). Relative to MDT-1, *C. butyricum* had a smaller effect on the butyrate production by fecal microbes than MDT-1 (Table 1). It is conceivable that *C. butyricum* merely passed through the intestine without growing, and therefore had little effect on the composition of intestinal microflora. It was reported that administration of *C. butyricum* MIYARI 588 did not change butyrate concentration in the intestinal contents of rats (16). Thus, MDT-1 may be more effective than *C. butyricum* in preventing colorectal cancer.

A number of studies indicate that administration of bifidobacteria or lactobacilli alone, or with fermentable carbohydrates including prebiotics, can alter colonic microflora populations and decrease the development of early preneoplastic lesions and tumors in the colon (53). Rafter (47) hypothesized that the carcinostatic effects of these bacteria may be in altering the metabolic activities of intestinal microflora (reducing the activity of β-glucuronidase, nitroreductase and azoreductase), altering physicochemical conditions in the colon such as pH (preventing the growth of putrefactive bacteria), binding or degrading potential carcinogens, producing antimutagenic or antimutagenic compounds, enhancing the host’s immune response, and modifying host physiology. The mechanisms by which these bacteria inhibit colon cancer remain to be clarified.

In conclusion, oral administration of the live *B. frigorolvens* MDT-1 cells to mice suppressed ACF formation. The mechanisms underlying this event are presently unclear, but may involve a decrease in β-glucuronidase activity in the colorectal contents, increases in the numbers of NK and NKT cells, and perhaps enhanced butyrate production in the gut. It may be possible to prevent or suppress colorectal cancer in humans and pet animals by the utilization of MDT-1 as a probiotic.

**LITERATURE CITED**


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