Effects of Intake of Pickles Containing Lactobacillus brevis on Immune Activity and Bowel Symptoms in Female Students

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Summary Forty-four female students with a tendency for constipation (mean age, 20.2±3.3 y) were asked to consume 30 g test pickles daily for 2 wk and were divided into 3 groups: viable-cell intake subjects (n=14, 3.0×10⁵ colony-forming units of viable LAB (lactic acid bacteria) cells per sample), dead-cells intake subjects (n=15, viable cells were heat sterilized), and placebo-intake subjects (n=15, LAB removed from the pickles). γ-Aminobutyric acid content of 75.1±3.2 mg per sample was noted, with no marked difference between samples containing viable and dead cells. Natural killer (NK)-cell activity (% specific lysis) in serum from dead-cell intake subjects was 37.5±17.0% before the start of the test-food intake and 47.7±20.1% after intake, indicating statistically significant effects (p<0.01). However, viable-cell intake and placebo intake subjects showed no statistically significant difference. The number of days with bowel movements significantly increased from 3.8±1.5 to 4.9±1.8 d in the dead-cell intake group, whereas a slight change from 4.6±1.5 to 5.1±1.7 d was observed in the viable-cell intake group. Additionally, the feeling of incomplete evacuation fell and a refreshed feeling increased among the subjects with constipation. Thus, marked enhancement of NK-cell activity and improved bowel symptoms were observed in subjects consuming pickles containing dead LAB cells.

Key Words lactic acid bacteria, natural killer activity, constipation, bowel symptoms, γ-aminobutyric acid

In recent years, concern has been raised about the increased risk of colorectal cancer related to intestinal environmental degradation, mainly among young people, in the context of the spread of westernized lifestyles characterized by decreased carbohydrate intake and increased intake of lipids and animal proteins (1, 2). Intestinal tissues absorb nutrients into the body and serve as the first line of defense against pathogens or foreign substances. The intestine, in which as much as 50% to 60% of total peripheral lymphocytes are concentrated, acts as a digestive system as well as the largest immune organ in the human body (3). Although eating 3 regular meals a day and keeping bowel movements regular are essential for a healthy lifestyle, constipation is difficult to cure completely owing to its variety of complicated causes (4). Constipation is a serious problem, especially in elderly people and patients with mental disorders (5–7). Foods that can be eaten continually along with 3 regular meals a day should preferably offer health-promoting functions such as improvement of bowel symptoms.

Commercially available LAB (lactic acid bacteria) beverages or soybean oligosaccharide foods that claim

to improve the intestinal environment have been developed and are enriched in dietary fibers that provide an intestinal-regulating effect as well as physiologically active amino acids, vitamins, and γ-aminobutyric acid (GABA) (8–10). However, these food products can be considered supplements and have been associated with adverse health effects depending on the type or concentration of their components (11).

Conversely, pickles are eaten frequently in Japan and have been recognized as safe. They are low in energy density (40–50 kcal per 100 g) and considered ideal foods for supplying minerals or vitamins (12). They are also considered health foods that improve constipation because they contain 2% insoluble dietary fibers, which can stimulate intestinal peristalsis (12).

As part of a dietary education effort in universities promoted by the Ministry of Education, Culture, Sports, Science and Technology, a questionnaire was administered to female freshmen and sophomores who consumed GABA-containing pickles fermented with Lactobacillus brevis subsp. (house strain, named LAB NSB2) as part of an adequate diet. The results showed improvement in bowel symptoms in students with constipation (13). The process suggested that heat-sterilized pickles improved bowel symptoms in subjects with constipation.

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and that the effect might not be due to the GABA in the pickles but to the dead LAB themselves. LAB sterilized by heat have been reported to enter the bloodstream through the intestine to increase and improve immunity by supporting leukocytes even though LAB are excreted in feces after being active in the gastrointestinal tract while alive (14). This study examined the effects of live or dead LAB contained in pickles on bowel symptoms in subjects with constipation and measured changes in immune activity (natural killer (NK) cells) and immunoglobulin (Ig) M and IgG levels in serum.

METHODS

Subjects. All of these procedures were conducted with the approval of the research ethics committee of Mukogawa Women’s University. We placed subjects in the constipation or non-constipation group using the following criteria. Subjects were placed in the constipation group if they (1) had hard stools, difficulty in having a bowel movement, and rare bowel movements (approximately 1 in several days), or (2) had daily bowel movements with hard stools and a feeling of residual stool or distressing bowel movements. Subjects were placed in the non-constipation group (54 volunteers), if they had bowel movements only once every 2 to 3 d but had normal stools and did not find bowel movements distressing. The non-constipation group was included only in the study of bowel symptoms; blood collection studies were omitted.

Table 1 indicated the comparison among anthropometric measurements of participants. Forty-nine and 58 female volunteers with and without constipation, respectively, provided written informed, as described above. Five volunteers in the constipation group were excluded for prematurely discontinuing intake during the study or refusing blood collection, leaving 44 healthy university freshman or sophomore volunteers aged 18 to 21 y with a tendency for constipation in the constipation group. There were found between constipation and non-constipation groups, although the weekly frequency of bowel movements was significantly lower in the constipation group.

Indicator bacteria in test food samples and bacterial cell counting. LAB NSB2 was passaged at 34˚C in glycerol, yeast extract, and peptone (GYP) basal medium (15). LAB, total viable bacteria, and Escherichia coli were selected as the indicator bacteria in test food samples. For cell counting, bacteria were incubated for 48 h at 34˚C using bromocresol purple plate count agar for lactic acid bacteria, standard agar for total viable count, and desoxycholate agar for Escherichia coli. Colonies (viable cells) grown on agar plates were counted as colony-forming units (CFUs) and expressed as the mean log-transformed CFU value±standard deviation (log CFU/30 g of sample) (13).

Preparation of test food samples. Three types of samples were prepared: samples containing viable cells, samples containing dead cells, and placebo samples. Test foods consisting of 30 g turnip pickles per subject as a 1-serving packaged unit were prepared as follows: (1) A total of 1.480 g turnip with the skin and cap removed and 75 g long green onion were soaked in a 5-fold volume of sterilized salt solution (0.85% NaCl) for 24 h at 4˚C. After draining, these foods were mixed with 740 g sterilized preparation of flavored soup (42.2 g dried bonito extract, 21.5 g dried kelp extract, 10.4 g dried sardine extract, and 666 g H 2 O) and refrigerated overnight. These foods were cut into 30-g pieces, enclosed in sterilized, heat-resistant, film-sheet bags, heated for 20 min at 80˚C, and used as preliminarily pickled turnips.

(2) NSB2 was incubated in GYP basal medium for 24 h at 34˚C and added to the preliminarily pickled turnips prepared in step 1 to a 1.0% (v/v) concentration and incubated for 48 h at 34˚C. Incubated turnips obtained via filtration with cheesecloth were soaked in sterilized vegetable-based seasoning liquid (containing 7.4% grated ginger, 3.7% sweet cooking rice wine, 44.9% soy sauce, 3.5% lemon juice, and 37.5% grated turnip) for 24 h and used as samples containing dead cells.

(3) After being sterilized via heating for 20 min at 121˚C, incubated turnips prepared in step 2 were soaked in sterilized vegetable-based seasoning liquid for 24 h and used as samples containing dead cells.

(4) After being sterilized via heating for 20 min at 121˚C, preliminarily pickled turnips prepared in step 1 were soaked in sterilized vegetable-based seasoning liquid for 24 h and used as placebo food samples.

Determination of GABA content. Test food samples/placebo food samples were analyzed for GABA content by using a Shimadzu chromatograph LC-9A amino acid analyzer equipped with Shin-pack Isc-07Na (Shimadzu Corporation, Kyoto, Japan) (13).

Distribution and intake of test foods, counting of indicator bacteria, and blood collection. The present study was a 4-wk, parallel-group study with 3 types of pickles as test foods. We included a screening period of 1 wk, a test food-intake period of 2 wk, and a follow-up period of 1 wk (13). A 3-d supply (including reserves) of packaging units of each test food was delivered by hand to subjects at 0900 on days 1, 4, 7, and 10. Reserves of packaging units of test foods were retained for examination and counting of indicator bacteria.

Subjects were provided with cooler bags and instructed to come to the laboratory for test foods. During the test food-intake period, subjects consumed 1 package of test foods consisting of 30 g turnip pickles per subject as a 1-serving packaged unit. This study examined the effects of live or dead LAB contained in pickles on bowel symptoms in subjects with constipation and measured changes in immune activity (natural killer (NK) cells) and immunoglobulin (Ig) M and IgG levels in serum.

Table 1. Baseline characteristics of subjects.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Constipation</th>
<th>Non-constipation</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of subjects</td>
<td>44</td>
<td>54</td>
</tr>
<tr>
<td>Age (y)</td>
<td>20.2±3.3</td>
<td>20.2±0.4</td>
</tr>
<tr>
<td>Body weight (kg)</td>
<td>53.4±7.0</td>
<td>54.1±7.4</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>157.2±5.1</td>
<td>158.7±5.8</td>
</tr>
<tr>
<td>Body mass index</td>
<td>21.5±2.4</td>
<td>21.4±2.6</td>
</tr>
<tr>
<td>Body fat percentage (%)</td>
<td>28.8±5.6</td>
<td>28.5±4.9</td>
</tr>
</tbody>
</table>

Values are means±SD.
foods once daily with a meal. Subjects were instructed not to change their previous daily life, including eating habits and physical activities, except for the daily intake of test foods. Blood samples were collected on the day of the first delivery of test foods (day 1) and the day of the last intake of test foods (day 14). During the follow-up period, dietary guidance was provided to subjects by physicians or a registered dietician.

**Examinations and statistical analysis.** Height was measured 1 wk before test food intake only. Body composition analysis was performed using InBody 3.2 (Biospace, Tokyo, Japan) at 2 time points—i.e., 1 wk before test food intake and at the end of the second week of test food intake. A food frequency questionnaire was administered to collect information such as content of meals, eating habits, and living conditions. This information was analyzed using Excel Eiyo-kun Ver. 2 FFQg software (Kenpakusha, Tokyo, Japan) (16). Subjects were asked to record bowel movements and stool characteristics on a daily basis. Fasting blood samples were collected at 2 time points: on day 1 of test food intake and at the end of 2 wk of test food intake. The data were analyzed to determine NK activity and IgM and IgG levels.

**Determination of NK cell activity.** Target cells were labeled with $^{51}$Cr and incubated with lymphocytes of 2 wk of test food intake. The data were analyzed to time points: on day 1 of test food intake and at the end of the second week of test food intake. A food frequency questionnaire was administered to collect information such as content of meals, eating habits, and living conditions. This information was analyzed using Excel Eiyo-kun Ver. 2 FFQg software (Kenpakusha, Tokyo, Japan) (16). Subjects were asked to record bowel movements and stool characteristics on a daily basis. Fasting blood samples were collected at 2 time points: on day 1 of test food intake and at the end of 2 wk of test food intake. The data were analyzed to determine NK activity and IgM and IgG levels.

**Determination of IgM levels.** A total of 3 µL serum was placed in a reaction cell, mixed with 300 µL R-1 buffer (Tris-HCl:N-assay TIA IgM-SH Nittobo kit, Nittobo Medical Co., Ltd., Tokyo, Japan), stirred, and kept warm for 5 min. The sample was then mixed and reacted with 50 µL anti-human IgM goat serum for 5 min while stirring, and turbidity was measured at 700 nm using an AU5400 (Beckman Coulter, Inc., Brea, CA) (18).

**Determination of IgG levels.** A total of 3 µL serum was placed in a reaction cell, mixed with 350 µL R-1 buffer (Tris-HCl:N-assay TIA IgG-SH Nittobo kit, Nittobo Medical Co.), stirred, and kept warm for 5 min. The sample was then mixed and reacted with 100 µL anti-human IgG goat serum for 5 min while stirring, and turbidity was measured at 700 nm using an AU5400 (Beckman Coulter, Inc., Brea, CA) (18).

**Table 2. Baseline characteristics of subjects with constipation by type of test food.**

<table>
<thead>
<tr>
<th>Sample solution</th>
<th>Viable cell group</th>
<th>Dead cell group</th>
<th>Placebo group</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of subjects</td>
<td>14</td>
<td>15</td>
<td>15</td>
</tr>
<tr>
<td>Age (y)</td>
<td>20.0±1.0</td>
<td>20.2±1.3</td>
<td>20.4±1.4</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>154.4±4.6</td>
<td>159.3±4.3</td>
<td>157.8±4.8</td>
</tr>
<tr>
<td>Body weight (kg)</td>
<td>48.1±6.8</td>
<td>53.1±5.4</td>
<td>52.1±7.8</td>
</tr>
</tbody>
</table>

Values are means±SD.

**Table 3. Distribution of indicator bacteria, $\gamma$-aminobutyric acid (GABA) content, and salt content in reserves of test food samples.**

<table>
<thead>
<tr>
<th>Sample solution</th>
<th>pH</th>
<th>Escherichia coli (CFU/mL)</th>
<th>Total viable bacteria (CFU/mL)</th>
<th>LAB (CFU/mL)</th>
<th>GABA (mg/30 g)</th>
<th>Salt $^1$ (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Viable cells</td>
<td>4.2</td>
<td>&lt;10</td>
<td>2.70E+06</td>
<td>2.20E+06</td>
<td>75.1</td>
<td>1.5</td>
</tr>
<tr>
<td>Dead cells</td>
<td>4.4</td>
<td>&lt;10</td>
<td>&lt;10</td>
<td>&lt;10</td>
<td>72.6</td>
<td>1.3</td>
</tr>
<tr>
<td>Placebo</td>
<td>4.2</td>
<td>&lt;10</td>
<td>1.40E+02</td>
<td>1.00E+01</td>
<td>5.6</td>
<td>1.4</td>
</tr>
</tbody>
</table>

Data were averaged across 4 time points. CFU, colony-forming units.

$^1$ grams per 100 mL.
observed, except that the body weight in the viable cell intake group showed a tendency to be low (19). A systematic analysis was used for comparison of baseline subject characteristics between the constipation and non-constipation groups, and the Mann-Whitney U-test (19, 21) was used for comparison of feeling after bowel movements and changes in stool form associated with test food intake between the constipation and non-constipation groups.

RESULTS AND DISCUSSION

Indicator bacteria and cell counts in reserves of test food samples

In the process of fermentation by LAB NSB2, increased lactic acid bacteria and production of lactic acid were observed. As shown in Table 3, these changes were accompanied by a decrease in pH in samples and a substantial decrease in cell counts of coexistent E. coli. Therefore, samples incubated for 48 h to suit the test food-intake schedule were used as test foods. Microscopic examination showed that the majority of the colonies that formed on media for common viable bacteria exhibited a characteristic morphology consistent with L. brevis subsp. coagulans.

GABA and salt contents in test foods

The GABA content in test foods delivered at each of the 4 time points remained almost constant throughout the 2-wk study. The GABA content in the placebo food group was originally defined as the threshold in raw materials. The salt content, which might affect flavor sensation, was similar in both test foods (see Table 3). A preparation of flavored soup was added so that the odor of the GYP medium components in the samples would not affect sensory evaluation, but subjects did not appear to be particularly conscious of these components. By contrast, the addition of vegetable-based seasoning liquid had a positive effect on the maintenance of cell counts of LAB during storage at 4°C. When no vegetable-based seasoning liquid was added, cell counts in samples tended to decrease by 1 order of magnitude in 24 to 72 h (data not shown). The vegetable-based seasoning liquid created a favorable environment for salt content and the maintenance of pH levels.

Microbial flora in test foods

Escherichia coli count was below the limit of detection at all 4 time points. By contrast, LAB count ranged from 10^5 to 10^6 cells in samples containing viable cells, with no significant changes during storage at 4°C (see Table 3). The GABA contents in placebo food samples were affected by the GABA contents originally present in vegetable materials, which were only 5–7% of the amount obtained from the LAB culture. pH changes and salt content did not differ between the test food and placebo samples, suggesting that subjects were not conscious of these characteristics.

Effects of test food samples on feces form and feeling after bowel movements

The effect of test foods on the feeling after bowel movements and stool characteristics was evaluated (Fig. 1a). For fecal form, a slight increase in “banana-shaped + half paste” forms was noted overall, consistent with a tendency toward improvement. In the constipation group, all subjects consuming samples containing dead cells had “banana-shaped + half paste” stools, which is a healthy stool form. All subjects in the viable cell intake group experienced a change in stool form from “muddy + watery” stools before intake to normal “banana-shaped” stools after intake.

Comparison of the feeling after bowel movements indicated that the number of subjects with refreshed feeling had decreased in the viable cell intake group, whereas the number doubled in the dead cell intake group. No change was found among subjects with constipation in the placebo intake group, whereas a decrease was found among subjects without constipation. The number of subjects in both the constipation and the non-constipation groups feeling refreshed after bowel movements decreased in the viable cell intake group and increased in the dead cell intake group (Fig. 1b). Overall, no substantial change in fecal color occurred (Fig. 1c) and no subjects found their fecal odor “strong,” indicating a slight tendency toward improvement (Fig. 1d). This result, together with data showing that normal dark brown stools were observed in all subjects (Fig. 1c), indicated that dead cell intake had positive effects in inducing proper bowel movement behavior. This is because fecal odor indicates whether the intestinal environment is normal, i.e., beneficial bacteria such as bifidobacteria predominate in the microbial flora. Decreases in harmful bacteria (such as Welch bacilli), which are responsible for the unpleasant odors such as ammonia, in the intestinal environment were expected.

NK activity, IgG levels, IgM levels, and blood fatty acids

Figure 2 demonstrates that mean differences among the viable cell intake, dead cell intake, and placebo groups were tested using the Mann-Whitney U test, a non-parametric method, because normal distribution could not be assumed (19, 21). In the 15 subjects in the dead cell intake group, NK activity increased in 11 subjects, decreased in 3 subjects, and showed no change in 1 subject. Thus, statistically significant differences were found before and after dead cell intake (Fig. 2a). In contrast, no statistically significant differences were found in the medians before and after intake for subjects consuming viable cell samples or those consuming placebo samples, suggesting that test food intake was associated with the enhancement of NK-cell activity only in the dead cell intake group (22, 23).

In the 15 subjects in the viable cell intake group, IgG levels increased in 7 subjects and decreased in 8 subjects (Fig. 2b). In the 14 subjects in the dead cell intake group, IgG levels increased in 5 subjects and decreased in 9 subjects (see Fig. 2b). In the 15 subjects in the placebo group, IgG levels increased in 8 subjects and decreased in 7 subjects (see Fig. 2b).

In the 15 subjects in the viable cell intake group, IgM levels increased in 7 subjects, decreased in 7 subjects, and showed no change in 1 subject (see Fig. 2c). In the dead cell intake group, IgM levels increased in 6 subjects and decreased in 8 subjects (see Fig. 2c). In the placebo
IgM levels increased in 8 subjects, decreased in 6 subjects, and showed no change in 1 subject (see Fig. 2c). These results demonstrated that no statistically significant differences existed between F-value and F critical value (5%) in the IgG and IgM intake groups when compared with the Mann-Whitney U test (19, 21), indicating that sufficiently low results for p value and critical values (5%) were obtained (24).

Symptoms of constipation

Constipation occurs when stools remain in the large intestine for a long period, lose water, and become hard. As demonstrated in Table 4, in the non-constipation group, no change was identified in the number of days with bowel movements after test food intake (5.7±1.1 d before or 5.7±1.6 d after), whereas the constipation group showed a significant increase in the number of days with bowel movements after test food intake (4.5±1.6 d compared with 3.8±1.8 d before test food intake; p=0.037). The weekly frequency of bowel movements was significantly lower in the constipation group.

No significant differences in age, height, body weight, body mass index, or body fat percentage were found between the constipation and non-constipation groups.

When the Mann-Whitney U test was used, statistically significant differences in anthropometric measurements were found before and after dead cell intake (p<0.05). Moreover, no statistically significant differences were found in medians before and after intake were found among subjects consuming samples containing viable cells or placebo (25–27). Tests among the 3 groups (viable cell, dead cell, and placebo) showed no positive correlation.

As demonstrated above, in the constipation group, the incidence of very hard stools was decreased, and that of banana-shaped or half-paste stools, which represent an optimum stool form, were increased. The increase in muddy and watery stools, which are characteristic of diarrhea, is likely due to the effect of the dietary fiber found in the test food. In the constipation group, most subjects had a feeling of incomplete evacuation.
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The number of subjects with this feeling regardless of GABA content, the presence of cell bodies in pickles was associated with an increase in the number of days with bowel movements, an improvement in stool form, and improvement in the feeling after bowel movements, suggesting generally modest effects in improving constipation. However, most subjects had a feeling of refreshment after bowel movements before test food intake, whereas the number of subjects with a feeling of refreshment after bowel movements decreased after test food intake. Because subjects were instructed not to change lifestyles, including eating habits and physical activities, beyond the daily intake of test foods during the study, these changes were likely due to causes other than test food intake and remain unknown.

No appropriate supportive measures are available for the treatment of constipation, which is a condition with a variety of complicated causes (1). However, daily intake of pickles has been demonstrated to improve the environment inside the intestines of young women, indicating that continued intake is beneficial in easing constipation.

Stools are a result of normal postprandial metabolic processes and reflect the environment inside the human large intestine. Differences in lifestyle and dietary components are reflected in the bacterial flora of stools. One report has concluded that changes in Japanese dietary intake from more traditional Japanese foods to high-fat foods may substantially decrease the fecal bifidobacteria ratio and increase *Bacteroides* species (2). Continued intake of high-cholesterol meals increases *Welch bacilli* in the intestinal flora, which is accompanied by increases in **β-glucuronidase** and nitroreductase activities in

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**Fig. 1.** Effect of sample intake on feces form (a), feeling after bowel movements (b), feces color (c), and feces odor (d). The vertical line represents the distribution ratio (%) calculated using a paired t-test (19). Changes before and after test food intake are shown for both the constipation and non-constipation groups. Comparisons before and after sample intake (*p* < 0.05) were made using the Bonferroni multiple-comparison procedure (19, 21).
Table 4. Changes in the number of days with bowel movements.

<table>
<thead>
<tr>
<th>Group</th>
<th>Subgroup</th>
<th>Before intake (d)</th>
<th>After intake (d)</th>
<th>Change</th>
</tr>
</thead>
<tbody>
<tr>
<td>Viable cell group</td>
<td>Non-constipation</td>
<td>5.4±1.1</td>
<td>5.6±1.3</td>
<td>0.2</td>
</tr>
<tr>
<td></td>
<td>Constipation</td>
<td>4.6±1.5</td>
<td>5.1±1.7</td>
<td>0.5</td>
</tr>
<tr>
<td>Dead cell group</td>
<td>Non-constipation</td>
<td>5.8±1.0</td>
<td>5.9±1.0</td>
<td>0.1</td>
</tr>
<tr>
<td></td>
<td>Constipation</td>
<td>3.8±1.5</td>
<td>4.9±1.8</td>
<td>1.1*</td>
</tr>
<tr>
<td>Placebo group</td>
<td>Non-constipation</td>
<td>5.5±1.8</td>
<td>6.4±0.9</td>
<td>0.9</td>
</tr>
<tr>
<td></td>
<td>Constipation</td>
<td>4.0±1.0</td>
<td>3.7±1.5</td>
<td>−0.3</td>
</tr>
</tbody>
</table>

Data are expressed as means±standard deviation. *p<0.05.
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The β-glucuronidase in stools is almost identical to that produced by bacteria in the intestines and an E. coli-specific enzyme found in 95% of E. coli (28). Harmful bacteria such as Welch bacilli appear to have a role in eliminating good intestinal flora from conjugation with useful metabolites. They do so by inhibiting the maintenance of homeostasis in the intestinal epithelium by the intestinal flora (such as bifidobacteria) that antagonizes them (27). This effect is reportedly inhibited by the oral administration of Lactobacillus bacteria (26, 28). Such inhibition of pathogenic bacterial growth by probiotics may be induced by reducing the colonization of the intestinal epithelium with pathogenic bacteria (29, 30) and inhibiting the activity of β-glucuronidase produced by pathogenic bacteria while enhancing beneficial β-glucuronidase activity (28). By contrast, potent bacterial activity exhibited by a variety of physiologically active substances (bacteriocins) produced by lactic acid bacteria and decreases in environmental pH associated with the production of organic acids have been reported to inhibit the growth of harmful bacteria (29–31).

Pasteurization at 60–80°C, which is often conducted in food manufacturing settings to ensure food quality, can almost completely kill harmful gram-negative bacteria, but creates an environment that allows the growth of non-pathogenic general bacteria, especially lactic acid bacteria. Because lactic acid bacteria can grow even on medium for total viable count, the cell counts of total viable cells/lactic acid bacteria in test foods may have included L. brevis subsp. coagulans, which were added to raw materials and produced by incubation (see Table 3). After intake began, no statistically significant differences were found in GABA content, microbial flora or cell counts during the entire study (see Table 3). One report of health-promoting and health-protecting effects of GABA other than improvement of constipation has described pronounced increases in α-interferon levels in healthy adults who took in viable cells of L. brevis, although dead cells after heating had no beneficial effect (32). Shimada et al. (3) have found that the intake of GABA-containing chlorella had a beneficial effect on blood pressure in patients with hypertension.

In recent years, evidence has accumulated that dead bacterial cells can enhance immune activity in mammals, including humans (13, 14, 32, 33). LAB, which live in a symbiotic relationship with humans in the intestines, recognize pathogenic bacteria, viruses, and food allergens as foreign and mediate bodily immune functions to eliminate them—i.e., the system by which intestinal bacteria, which naturally live symbiotically in host intestines, defend against pathogenic bacteria present in foods that are consumed. We hope that the system by which intracellular components released from dead cells affect the intestinal tissues of their human hosts to produce physiological effects will be elucidated in the future (34–36).

Although the composition of intestinal flora varies according to the meal patterns of hosts, no consensus has been reached among researchers about the composition due to factors such as dietary components, eating habits, or age. A common concept in evidence accumulated to date is that dramatic changes in human intestinal flora are unlikely to occur unless extreme changes occur in the diet (1, 32, 37).

Increasing beneficial bacteria by improving the intestinal environment through diet modification is essential for health promotion (7). Increases in the numbers of Bifidobacterium bifidum or Enterococcus faecalis, the typical beneficial bacteria in the body, are likely to increase the levels of organic acids produced by these bacteria (such as lactic acid and acetic acid) and reduce the pH of the intestinal environment. Thus, the following are achieved: 1% or less harmful bacteria levels, decrease in putrefaction products such as ammonia to approximately one-third, and improvement in fecal odor (Fig. 1). In addition, stimulation of intestinal peristalsis has been associated with a decrease in the number of days with bowel movements (Fig. 1).

The positive effects of dead cell intake observed in the present study are likely due to the stimulation of immune cells by the cell wall components (such as polysaccharides) of dead cells in the intestine, leading to the activation of white blood cells. Bacteria need not be viable to provide such effects. On the contrary, the entry of viable cells directly into the intestine appears to be harmful for maintaining immune activity because it disrupts the balance of intestinal microbial flora (Figs. 1 and 2).

One study showed that dead cells alone serve as “fair game” for intestinal microbial flora and increase the E. faecalis, which is identified as a beneficial bacterium population (38). An additional advantage of dead cells is their low water content when compared with that of viable cells and the ability to introduce a correspondingly large number of cells (game) into the body.

The effects of the intake of pickles, which are rich in dietary fibers, may be diminished by the small effects of viable cells and placebo food. Intake of dead cells likely induced an increase in cell counts of endogenous beneficial bacteria and an associated decrease in putrefaction products, which led to enhanced immune activity, beneficial effects on intestinal peristalsis, and finally, improved symptoms of constipation and enhancement of immune activity (39).

Notably, the present study did not make a quantitative assessment of stool consistency because it could not score hydration status and subjects visually assessed stool characteristics (40). In future studies, quantification of the improvement of bowel symptoms by intake of dead cells—preferably measurement of gut transit time—is necessary.

Findings from the present study indicate that metabolites released by dead cells of lactic acid bacteria may promote health and improve symptoms of constipation by stimulating the activation of human NK cells (37, 41). The advantages of using sterilized dead cells is that it allows for the intake of the dietary fiber supplements present in pickles at any time or place, potentially leading to improved bowel movements and enhanced immune activity.
We obtained GABA-producing LAB from Shibazuke pickles, a traditional food in Kyoto, Japan, and tentatively identified them as *Lactobacillus buchneri*. A detailed review of this strain using 16S ribosomal RNA analysis is now under way (42, 43).

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REFERENCES


