Application of Probiotics from Mongolian Dairy Products to Fermented Dairy Products and Its Effects on Human Defecation

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We screened suitable lactic acid bacteria (LAB) strains for use in fermented dairy products by analyzing milk fermented with 10 probiotic LAB strains from Mongolian dairy products. Lactobacillus paracasei paracasei strain 06TCa19 was selected because of its favorable effects on pH, lactic acid production, and viable bacterial numbers after fermentation of skim milk. Then, we prepared 06TCa19 and control fermented milks and conducted a randomized, double-blind crossover study with 46 healthy women to determine the effects of the strain on human defecation. The ingestion of 06TCa19 fermented milk improved the subjects’ fecal characteristics, including shape and color. Analysis of stool samples from 8 subjects revealed that \( l^-\)lactic acid levels and Lactobacillus and Bifidobacterium numbers increased. Moreover, strain 06TCa19 was suggested to reach and survive in the intestines, and is, therefore, suitable for fermented dairy products and can potentially improve human defecation.

Keywords: probiotics, Mongolian dairy product, Lactobacillus, fermented dairy products, human defecation

Introduction

Many studies have demonstrated the probiotic effects of lactic acid bacteria (LAB) and Bifidobacteria on human health in vitro and in vivo (Ishida et al., 2005; Kimura, 2004; Kumagai et al., 2004; Ohata et al., 2011; Xiao et al., 2003). The World Health Organization defines probiotics as living microorganisms that confer health benefits to the host when administered in adequate amounts (i). Probiotics can improve the host’s intestinal environment (Parvez et al., 2006). Nowadays, many people, even those considered healthy, are in a state of chronic stress, lack proper exercise, and complain of intestinal disorders such as constipation and diarrhea (Ohya and Yoneda, 1995). Therefore, maintaining good bowel habits through probiotic use is expected to improve peoples’ quality of life.

In the past decade, the microbiota and diversity of LAB species in Mongolian dairy products have been elucidated (Miyamoto et al., 2010; Takeda et al., 2011a; Uchida et al., 2007; Watanabe et al., 2008; Yu et al., 2011). Traditional Mongolian dairy products are consumed not only as important energy sources but also as traditional medicines (Watanabe, 2011). Therefore, further studies elucidating the functions and LAB content of dairy products are anticipated. Several studies also demonstrated the functions of LAB in Mongolian dairy products (Batdorj et al., 2006; Kimura et al., 2006; Takeda et al., 2006; Takeda et al., 2011b). However, few studies deal with the effects of probiotics from Mongolian dairy products on human defecation, an essential function of probiotics.

Previously, we isolated many strains from traditional Mongolian dairy products. Our results of tolerance tests to low pH and bile acid, as well as adhesion test using Caco-2 cells, indicate that several strains are expected to be probiotics (Takeda et al., 2011a). However, we did not demonstrate whether these candidate strains can reach and survive in the human intestine, and thus act as probiotics. In addition, it is
important for dairy product manufacturers to evaluate the growth of LAB strains in milk in order to apply the strains to their products.

In this study, we investigated the pH, lactic acid production, and number of viable bacteria in fermented milk among candidates from Mongolian dairy products, to select a suitable probiotic LAB strain for use in fermented dairy products. The selected LAB strain and control fermented milks were prepared and used in a randomized, double-blind crossover study to investigate the probiotic effects of the strain on human defecation.

Materials and Methods

LAB strains and milk fermentation test  Ten potential probiotic LAB strains isolated from Mongolian dairy products were used (Takeda et al., 2011a). For the skim milk fermentation test, the LAB were precultured at 37°C for 24 h in 5 mL de Man, Rogosa and Sharpe (MRS) broth (Merck, Darmstadt, Germany). Then, cells were washed twice with sterilized phosphate-buffered saline (PBS; pH 7.3) and suspended in 5 mL sterilized PBS. The LAB solutions were fermented at 37°C for 24 h. Curd formation in the fermented milk was observed visually. The colony forming unit (CFU) of the LAB strain in the fermented milk was measured with BCP agar (Nissui, Tokyo, Japan). The pH was measured with a pH meter (F-12, Horiba, Kyoto, Japan). Lactic acid concentration (%) was determined by titration with 0.1 N NaOH and calculated using the following equation:

\[
\text{Acidity (lactic acid [\%])} = \frac{0.9 \times \text{titration volume (mL)} \times \text{factor}}{\text{sample weight (g)}}
\]

Fermented milk preparation  We used Lactobacillus paracasei paracasei strain 06TCa19 (1.3 × 10⁸ CFU/g) in the test fermented milk with a pH of 4.38. Placebo fermented milk containing the non-probiotic Streptococcus thermophilus strain 510 (4.1 × 10⁸ CFU/g) with a pH of 4.43 was used as a control. Other than bacterial contents, both fermented milks were made of milk, defatted milk, milk whey protein, glucose, gelatin, and agar. The taste of the fermented milks was almost identical.

Study schedule and fecal sampling  A double-blind crossover study designed to investigate the effects of ingesting fermented milk containing strain 06TCa19 was conducted. Throughout the experimental period, the subjects regulated their diet to avoid eating other fermented products and products containing probiotics and oligosaccharides. Forty-six female students (18 − 39 years old) were randomly divided into 2 groups: those receiving fermented milk with probiotic or with nonprobiotic bacteria. Observations were made for 1 week before the intake periods to obtain baseline values for the test. During the first intake period, the subjects consumed 100 g of fermented milk twice a day (in the morning and evening) for 3 weeks. This was followed by a 1-week washout period, during which no fermented milk was consumed. Then, the subjects consumed the other fermented milk for another 3 weeks. At the end of each week of the experiment, fecal samples were collected in sterile packs under anaerobic conditions (AneroPack, Mitsubishi Gas Chemical, Tokyo, Japan) from the 8 (of 46) subjects who agreed to provide fecal samples. The fecal samples from each week were analyzed within 1 day of collection. All the examinations were performed in accordance with the guidelines of the Helsinki Declaration and the ethical committee of Minami Kyushu University, Japan, which approved this study (Permission No. 35).

Defecation records and fecal characteristics  Throughout the examination period, the subjects recorded their defecation frequency and quantity, and assessed their fecal characteristics using a score sheet. Defecation quantity was expressed in terms of the volume of table tennis balls, one of which was given to each subject as a reference. Fecal shape was described as 1 of 6 types, ranging from “watery” to “very hard”. Fecal color was described as 1 of 6 colors, ranging from yellow to black.

Analysis of fecal pH, and ammonia and l-lactic acid concentrations  At several points, the subjects were asked to directly measure fecal pH after defecation using a bromothymol blue pH test paper (Advantec, Tokyo, Japan). To measure ammonia and lactic acid concentrations, 1 g of feces was suspended in 9 mL of sterilized PBS (pH 7.3) at 4°C. The sample suspension was then centrifuged at 3000 rpm at 4°C, and the supernatant was collected and stored at −80°C until analysis. The supernatants were diluted with 2% perchloric acid, and the concentrations of ammonia and l-lactic acid were determined using an ammonia test kit (Wako Pure Chemical, Osaka, Japan) and a F-kit l-lactic acid (J.K. International, Tokyo, Japan), respectively.

DNA extraction from fecal samples and intestinal bacteria enumeration  DNA was extracted from fecal samples and standard strains using ISOFECAL for Beads Beating (Nippon Gene, Tokyo, Japan) according to the manufacturer’s instructions for quantification by real-time PCR. Standard strains were used for each group, including Bifidobacterium longum JCM 1217 for the Bifidobacterium subspecies, the strain 06TCa19 for the Lactobacillus group, Clostridium coccoideis JCM 1395 for the C. coccoideis group, and Bacteroides fragilis JCM 11019 for the Bacteroides-Prevotella group. Except for strain 06TCa19, these strains were cultured at 37°C for 24 − 48 h in GAM broth and on
GAM agar (Nissui) containing 0.5% glucose in jars with anaerobic packs for CFU enumeration. Meanwhile, strain 06TCa19 was cultured at 37°C for 24 h in MRS broth and on MRS agar for CFU enumeration. *Bifidobacterium* subspecies and *C. coccoides, Bacteroides-Prevotella, and Lactobacillus* groups were detected by specific SYBR Green real-time PCR. Specific primers and their annealing temperatures are shown in Table 1. Gene quantification was performed on an Applied Biosystems 7300 real-time PCR system (Applied Biosystems Japan, Tokyo, Japan). The reaction mixture (20 μL) contained 10 μL of 2 × SYBR Green Master Mix (Applied Biosystems Japan), with a total volume of 25 μL: 1.0 μL of bacterial DNA, 2.0 μL of dNTP mixture, 2.5 μL of 10 × buffer, 0.2 μL of 5 units/μL Taq polymerase (Takara Bio, Otsu, Japan) and subjected to random amplified polymorphic DNA (RAPD) PCR analysis. The primers for the RAPD PCR were 1254 (5′-CCGCAGGCCAA-3′; Akopyanz et al., 1992) and AT41 (5′-CGGATGTGTTT-3′; Takeda et al., 2011a). The PCR reaction was performed in a DNA thermal cycler (GeneAmp PCR System 2700, Applied Biosystems Japan), with a total volume of 25 μL: 1.0 μL of bacterial DNA, 2.0 μL of dNTP mixture, 2.5 μL of 10 × buffer, 0.2 μL of 5 units/μL Taq polymerase (Nippon Gene), 0.5 μL of each primer (100 μmol/L), and 18.8 μL of purified water. The cycling program for primer 1254 consisted of 4 cycles of 94°C for 5 min, 36°C for 5 min, and 72°C for 5 min; 30 cycles of 94°C for 1 min, 36°C for 1 min, and 72°C for 2 min; and a final extension at 72°C for 10 min. The cycling program for primer AT41 consisted of an initial denaturation step at 94°C for 4 min; 40 cycles of 94°C for 1 min, 37°C for 1 min, and 72°C for 2 min; and a final extension at 72°C for 5 min. RAPD products were electrophoresed at 100 V on a 2.0% agarose gel including 0.3 μg/mL of ethidium bromide. The amplified DNA bands from 06TCa19 and other colonies were visualized by ultraviolet irradiation (254 nm) and photographed using an AE-6911FXN print graph (ATTO, Tokyo, Japan).

**Statistical analysis** The results regarding defecation frequency and quantity, and intestinal bacteria enumeration were statistically analyzed using one-way ANOVA, followed by the Tukey test and a two-tailed paired Student’s t-test. The χ² test was used to analyze the association between fecal characteristics and fermented milk consumption. The Mann-Whitney U-test was used to analyze fecal constituents. The level of significance was set at p < 0.05.

<table>
<thead>
<tr>
<th>Target bacteria</th>
<th>Amplicon size (bp)</th>
<th>Annealing temperature (°C)</th>
<th>Sequence (5′−3′)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Lactobacillus</em> group</td>
<td>341</td>
<td>58</td>
<td>F:AGCAATGGGGATCTTCCA R:CCCCCTACACATGGGAG</td>
<td>Rinttilä et al. (2004)</td>
</tr>
<tr>
<td><em>Clostridium coccoides</em> group</td>
<td>440</td>
<td>50</td>
<td>F:AAATGAGCGGTACCTGACTAA R:TTTGAGGTTTATCCGAGGAA</td>
<td>Matsuki et al. (2004)</td>
</tr>
<tr>
<td><em>Bacteroides-Prevotella</em> group</td>
<td>418</td>
<td>59</td>
<td>F: GAAGGTCGCCACATTTG R:CAATCGGAGTTTCTCGTG</td>
<td>Xu et al. (2011)</td>
</tr>
</tbody>
</table>

Table 1. Real-time PCR primers used in the present study.
Results and Discussion

Selection of LAB strains for use as probiotics in dairy products To select a suitable LAB strain for the fermentation of milk among the 10 strains, we investigated the curd formation, pH, lactic acid concentration, and numbers of viable bacteria in skim milk fermented by each strain (Table 2). The skim milks fermented by *L. paracasei paracasei* 06TCa19, *L. paracasei tolerans* 06TCa39, and *L. delbrueckii lactis* 06TC3 formed curds and exhibited reduced pH values and increased lactic acid concentrations. In particular, strain 06TC3 substantially reduced the pH and increased the lactic acid concentration. Meanwhile, strain 06TCa19 produced the greatest number of viable bacteria among all the strains. In contrast, only minimal changes were observed in milks fermented with *L. plantarum*.

The probiotic effects of many LAB strains have been demonstrated. In particular, *L. paracasei*, *L. casei*, and *L. rhamnosus*, which are genetically related species, are species popularly used as probiotic LAB (Collins et al., 1991). Oral administration tests in humans demonstrated that several strains identified as belonging to these species have a strong ability to survive in and colonize the human gut and improve the human intestinal microflora (Matsumoto et al., 2006; Nishida et al., 2008; Verdenelli et al., 2011). The present study identified 2 probiotic candidate strains, 06TCa19 and 06TCa39, belonging to *L. paracasei* that could grow well in skim milk. It is important to grow and maintain viable bacterial numbers in milk. The use of new probiotic LAB strains in dairy products, especially yogurt, requires adequate bacterial growth and maintenance of viable bacterial numbers in milk. The results of the milk fermentation test of these strains demonstrated that strain 06TCa19 showed lower pH, higher lactic acid concentration, and more viable bacterial number than those of strain 06TCa39. Additionally, the flavor of the fermented milk prepared using strain 06TCa19 was more favorable than that of strain 06TCa39 in a lab-scale trial (data not shown). These results indicate that strain 06TCa19 is more suitable for developing dairy products than strain 06TCa39. Therefore, strain 06TCa19 was used in the test fermented milk for oral administration tests in humans.

Effects of strain 06TCa19 on human defecation frequency and fecal characteristics The 06TCa19 and control fermented milks were prepared and given to healthy female subjects in a placebo-controlled, double-blind crossover test. No subjects dropped out owing to ill health. The defecation frequencies and quantities of the 06TCa19 and control groups are shown in Table 3. In both groups, the defecation frequencies and quantities during intake week 1 were significantly greater than those during the baseline week (*p* < 0.05). However, there were no significant differences in the defecation frequencies or quantities between the groups at any week according to the two-tailed paired Student’s *t*-test. Since the subjects recruited did not have any problems associated with their bowel habits, it was difficult to detect the effects of strain 06TCa19 on defecation frequency and quantity in this study. Actually, the values of defecation frequency and quantity for the baseline weeks in both groups seemed to be comparatively high. It is well known that ingestion of any fermented milk (with or without probiotics) improves the intestinal environment of humans. The observed increases in these parameters during intake week 1 in both the groups could be due to the potential effects of fermented milk on intestinal regulation.

In addition, the self-reported numbers of each fecal...
reported that ingestion of fermented milk with *Lactobacillus johnsonii* strain La1 increased the subjects’ fecal concentration of short chain fatty acids, including lactic acid. They also described that the intestinal acidic condition produced by strain La1 contributed to improved intestinal microflora and fecal characteristics. In this study, the proportion of “soft + banana shape” feces increased and that of “hard + very hard” feces decreased from the baseline week to intake week 1 in the fecal shape of the 06TCa19 fermented milk group. Fecal color became brighter from the baseline week to intake week 1, and the number of “yellow + brownish yellow” also increased. Meanwhile, no significant differences in these fecal condition were compiled (Table 3), and the relationships between the distribution of each fecal characteristic and ingestion of 06TCa19 or control fermented milk during the baseline week and intake week 1 were analyzed using the *χ²* test. There were significant differences in fecal shape and color in the 06TCa19 group between the baseline and intake week 1 (*p* < 0.05). Feces ideally have a “banana” shape (Takiguchi et al., 1997). Furthermore, Seki et al. (2004) described that the intestinal acidic conditions due to Bifidobacteria activity make human feces brightly colored, whereas alkaline conditions due to ammonia-producing bacteria produce darker feces. On the other hand, Yamano et al. (2004) reported that ingestion of fermented milk with *Lactobacillus johnsonii* strain La1 increased the subjects’ fecal concentration of short chain fatty acids, including lactic acid. They also described that the intestinal acidic condition produced by strain La1 contributed to improved intestinal microflora and fecal characteristics. In this study, the proportion of “soft + banana shape” feces increased and that of “hard + very hard” feces decreased from the baseline week to intake week 1 in the fecal shape of the 06TCa19 fermented milk group. Fecal color became brighter from the baseline week to intake week 1, and the number of “yellow + brownish yellow” also increased. Meanwhile, no significant differences in these fecal characteristics were observed with respect to shape and color in the 06TCa19 group between the baseline and intake week 1 but not observed in the control group by the *χ²* test (*p* < 0.05).

**Table 3.** Effects of strain 06TCa19 on human defecation frequency and quantity, and fecal characteristics.

<table>
<thead>
<tr>
<th></th>
<th>06TCa19 group (n = 46)</th>
<th>Control group (n = 44)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline week</td>
<td>Intake week 1</td>
</tr>
<tr>
<td><strong>Frequency</strong> (times/week)</td>
<td>5.6 ± 2.3</td>
<td>6.6 ± 2.9*</td>
</tr>
<tr>
<td><strong>Quantity</strong></td>
<td>18.7 ± 9.6</td>
<td>22.5 ± 13.2*</td>
</tr>
<tr>
<td><strong>Shape</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Watery + muddy</td>
<td>13 (5.5%)</td>
<td>27 (10.7%)</td>
</tr>
<tr>
<td>Soft + banana shape</td>
<td>154 (65.8%)</td>
<td>175 (69.4%)</td>
</tr>
<tr>
<td>Hard + very hard</td>
<td>67 (28.6%)</td>
<td>50 (19.8%)</td>
</tr>
<tr>
<td>Total</td>
<td>234 (100%)</td>
<td>252 (100%)</td>
</tr>
<tr>
<td><strong>Color</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yellow + brownish yellow</td>
<td>12 (5.2%)</td>
<td>32 (12.8%)</td>
</tr>
<tr>
<td>Brown + tea brown</td>
<td>139 (60.7%)</td>
<td>145 (58%)</td>
</tr>
<tr>
<td>Dark brown + black</td>
<td>78 (32.1%)</td>
<td>73 (29.2%)</td>
</tr>
<tr>
<td>Total</td>
<td>229 (100%)</td>
<td>250 (100%)</td>
</tr>
</tbody>
</table>

* Significantly different from baseline by one-way ANOVA followed by the Tukey test (*p* < 0.05).

**Table 4.** Effects of strain 06TCa19 on human fecal properties.

<table>
<thead>
<tr>
<th></th>
<th>06TCa19 group</th>
<th>Control group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline week</td>
<td>Intake week 1</td>
</tr>
<tr>
<td><strong>pH</strong></td>
<td>7.0 ± 0 (n = 8)</td>
<td>7.0 ± 0 (n = 8)</td>
</tr>
<tr>
<td><strong>NH₃ (mg/g)</strong></td>
<td>3.9 ± 1.6 (n = 8)</td>
<td>2.8 ± 1.4 (n = 7)</td>
</tr>
<tr>
<td><strong>l-lactic acid (μg/g)</strong></td>
<td>33.1 ± 15.2 (n = 8)</td>
<td>65.7 ± 39.2 (n = 8)</td>
</tr>
</tbody>
</table>

The numbers are expressed as mean ± standard deviation. The numbers in parentheses indicate the number of specimens.

* Statistically significant difference between the 06TCa19 and control groups during intake week 2 using the Mann-Whitney *U* test (*p* < 0.05).
cal characteristics were observed in the control group. From these results, it was suggested that 06TCa19 fermented milk could improve bowel habits better than the control product. The improvement of fecal characteristics may be due to the increased concentration of organic acids, including lactic acid, in the intestines produced by strain 06TCa19, similar to that observed for strain La1.

**Analysis of fecal properties** The results of fecal pH, and ammonia and 1-lactic acid concentrations are shown in Table 4. No notable changes were observed in fecal pH or ammonia concentrations in either group. However, during the fermented milk intake period, the 1-lactic acid concentration tended to be higher in the 06TCa19 group than the control group. In particular, during intake week 2, the 1-lactic acid concentration was significantly higher in the 06TCa19 group than the control group (p < 0.05). Strain 06TCa19 was confirmed to produce 1-lactic acid (data not shown). Thus, the increase in 1-lactic acid concentration in the feces of the 06TCa19 group is likely due to the presence of strain 06TCa19 in the subjects' intestine. Organic acids, including lactic acid, are thought to promote gastrointestinal motility (Yokokura et al., 1977). Therefore, the increased 1-lactic acid concentration in the intestine after the consumption of 06TCa19 fermented milk might have affected human fecal characteristics. These data also support our hypothesis that the increase in lactic acid concentration in the feces of the 06TCa19 group resulted in improved fecal characteristics, as mentioned above.

**Enumeration of human intestinal bacteria** The results of the enumeration of intestinal bacteria by real-time PCR are shown in Fig. 1. The total bacteria counts measured by DAPI staining did not differ between 06TCa19 and control groups and remained almost unchanged throughout the experiment in both groups. The 06TCa19 strain significantly increased Lactobacillus levels during the intake period compared with the baseline week (p < 0.05). Strain 06TCa19 was confirmed to produce 1-lactic acid (data not shown). Thus, the increase in 1-lactic acid concentration in the feces of the 06TCa19 group resulted in improved fecal characteristics, as mentioned above.

![Fig. 1. Intestinal bacteria numbers in fecal samples from subjects.](image-url)

(A) shows intestinal bacteria numbers in fecal samples from the subjects ingesting fermented milk containing *Lactobacillus paracasei* paracasei strain 06TCa19 (Test fermented milk). (B) shows those of the subjects ingesting fermented milk containing *Streptococcus thermophilus* strain 510 (placebo fermented milk). Fecal samples were collected weekly from 8 subjects. The black bars indicate the bacterial numbers during the baseline week. The dark gray bars show the bacterial numbers during intake week 1. The light gray bars show the bacterial numbers during intake week 2. The white bars show the bacterial numbers during intake week 3. The values are expressed as mean ± standard deviation. The asterisks indicate significant differences from the baseline by one-way ANOVA followed by the Tukey test (p < 0.05).
results from the culture method (data not shown). *C. coccoides* levels in the 06TCa19 group were significantly lower at intake week 1 than at the baseline \((p < 0.05)\), but recovered at intake week 2. Although no significant differences were observed, *Bifidobacterium* levels increased gradually and *Bacteroides-Prevotella* class levels decreased in a week-dependent manner in the 06TCa19 group but not the control group. Improved frequency of defecation and fecal quality in humans after ingestion of probiotics is suggested to be due to changes in the intestinal microbiota, particularly increases of *Lactobacillus* and *Bifidobacterium* (Matsumoto et al. 2006; Ogata et al., 1997; Olivares et al. 2006; Verdenelli et al. 2011; Yamano et al. 2006). In the 06TCa19 group, because the *Lactobacillus* levels increased significantly and the *Bifidobacterium* levels tended to increase in a time-dependent manner during the intake period, they might be involved in the observed improvement of fecal characteristics.

**RAPD PCR analysis for detecting strain 06TCa19 in fecal samples**  
To confirm that strain 06TCa19 in the fermented milk reached the intestine of subjects and remained viable, we collected fecal samples from 8 subjects after the intake weeks and used modified LBS plates to isolate 72 colonies from the samples. Twelve of the 72 colonies were randomly subjected to genotyping by RAPD PCR analysis (lanes 4 – 15) in addition to pure cultures of 06TCa19 (lane 1), 510 (lane 2), and *L. acidophilus* ATCC 43121 strains (lane 3; Fig. 2). All the genotypes were observed while using primer 1254; the 9 genotypes observed while using primer AT41 (lanes 4, 6 − 9, 11, 12, 14, and 15) were consistent with that of the pure culture of strain 06TCa19. Therefore, 9 genotypes (lanes 4, 6 − 9, 11, 12, 14, and 15) of the 12 randomly chosen *Lactobacilli* colonies from the subjects’ fecal samples were likely homologs of strain 06TCa19. These results suggest that 06TCa19 can reach and survive in the human intestine. They also indicate that the increase in fecal \(\alpha\)-lactic acid and changes in intestinal bacteria in the 06TCa19 group were due to live 06TCa19 in the intestine. Therefore, the improvement of human fecal characteristics through increased \(\alpha\)-lactic acid concentration in the intestine and the effects on intestinal bacteria, especially increased *Lactobacillus* and *Bifidobacterium* numbers, would be attributable to live 06TCa19 bacteria.

![Fig. 2. RAPD PCR profiles of the Lactobacilli isolates from subjects’ fecal samples.](image)

(A) shows RAPD PCR profiles of the randomized *Lactobacilli* isolates from the subjects’ feces detected by using primers 1254. (B) shows those of the subjects’ feces detected by using primers AT41. Lane M, lane 1, lane 2, lane 3, and lanes 4 – 15 are the DNA size marker, *Lactobacillus paracasei paracasei* strain 06TCa19, *Streptococcus thermophilus* strain 510, *L. acidophilus* ATCC 43121, and randomly selected *Lactobacilli* isolates from the feces of 8 subjects, respectively.
Conclusion

Strain 06TCa19 has potential as a probiotic in dairy products because of its beneficial effects on pH, lactic acid concentration, and the number of viable bacteria after skim milk fermentation. We prepared 06TCa19 fermented milk for a randomized, double-blind crossover study with 46 women who consumed either 06TCa19 or control fermented milk. Ingestion of 06TCa19 fermented milk improved the subjects’ fecal characteristics by increasing the l-lactic acid concentration in the intestine and modifying intestinal bacteria. Furthermore, RAPD analysis of Lactobacilli from the fecal samples indicated that strain 06TCa19 can reach the intestine and remain viable. In future, strain 06TCa19 is expected to be used as a probiotic in dairy products, especially yogurt.

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References


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