Survival of *Bifidobacterium animalis* subsp. *lactis* OPB-1 in the Gastrointestinal Tract after its Administration in a Milk-free Soybean Product and the Effect on Fecal Microbiota in Healthy Adults

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The survival of *Bifidobacterium animalis* subsp. *lactis* OPB-1 in the gastrointestinal tract after its administration in a milk-free yogurt-like soybean product and effects on fecal microbiota in humans were determined. Twelve healthy volunteers were administered 100 g of yogurt-like soybean product for 3 weeks. The plate count method combined with specific PCR and quantitative real-time PCR were used to estimate the numbers of total *Bifidobacterium* and *B. animalis* subsp. *lactis* in fecal samples. *B. animalis* subsp. *lactis* was detected during the administration period but not before. The intake number of *B. animalis* subsp. *lactis* OPB-1 was $2.4 \times 10^{10}$ CFU/day by the plate count method and $4.2 \times 10^{13}$ copies/day by quantitative real-time PCR. However, the number of excreted *B. animalis* subsp. *lactis* per day was estimated to be more than 10 times the intake number, by both methods. The percentage of *Bifidobacterium* increased from $16.9 \pm 21.8\%$ to $34.1 \pm 20.4\%$ by the plate count method and $18.9 \pm 13.4\%$ to $27.8 \pm 15.8\%$ by the T-RFLP method after 3 weeks administration.

Keywords: *Bifidobacterium animalis* subsp. *lactis*, fecal microbiota, soybean, T-RFLP, PCR

Introduction

Many bacteria live and form intestinal microbiota in the digestive tract of humans. The intestinal microbiota consists of more than 500 bacterial species and the number of bacteria is said to be $10^{11}$ per g of wet feces (Ohashi, 2006). The intestinal microbiota is believed to be closely related to host health (Mitsuoka, 1982). Among these, bifidobacteria are major intestinal bacteria, comprising 75 – 91% of intestinal bacteria in the digestive tract of infants (Harmsen *et al.*, 2000) and 3 – 7% of those of adults (Biavati and Mattarelli, 2001). Bifidobacteria are believed to contribute to health maintenance in hosts. These findings have shed light on the use of food products containing certain species of bifidobacteria, such as fermented milk products, as probiotics. A probiotic is defined as a live microorganism that exerts a desirable effect on the host when an optimal amount is ingested (Fuller, 1991). It has been reported that the ingestion of probiotics could be expected to improve the intestinal microflora (Fuller and Gibson, 1997), aid in bowel movement (Mori *et al.*, 2009), heighten immune function (Yasui *et al.*, 1992, Yasui *et al.*, 1995), prevent pollinosis (Odamaki *et al.*, 2007), and ameliorate irritable bowel syndrome (O’Mahony *et al.*, 2005). However, many probiotics are milk-based products, which people with milk allergy cannot consume. In addition, vegans cannot consume these products. Furthermore, the ingestion of lactic acid bacteria and bifidobacteria from fermented milk and the like entails the ingestion of milk fat and cholesterol, which is not considered desirable for everyone. In recent years, soybean-based yogurt-like food products have been produced. However, milk components are often used for the production of lactic acid bacteria and bifidobacteria. There are few products that are completely free of milk components. Several reports exist on the delivery of lactic acid bacteria and bifidobacteria contained in fermented milk to the intestine, and the ameliorating effects on the intestinal microbiota. However, there is scant information on soybean-based yogurt-like products free from milk components. *Bifidobacterium animalis* subsp. *lactis* is the best known probiotic, and is used in fermented milk (Biavati *et al.*, 1992) and

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drugs for controlling intestinal functions (Salminen et al., 2005).

We prepared a yogurt-like soybean product free from milk components, using B. animalis subsp. lactis OPB-1 previously cultivated in milk-free media. The objectives of this study were to show that B. animalis subsp. lactis OPB-1 passes live through the digestive tract and to examine its effect on intestinal microbiota, as the first step in characterizing the benefits of milk-free yogurt-like soybean products. The behavior of B. animalis subsp. lactis in the digestive tract is also discussed.

Materials and Methods

Bacterial strains and yogurt-like soybean product (YSP) B. animalis subsp. lactis OPB-1 was provided by Sacco Inc. (Cadorago, Italy) as a concentrated frozen pellet (10^{11} CFU/g). This strain had been cultivated in a milk component-free medium, and was maintained at −80°C before use. Pediococcus pentosaceus IDS885m4 was grown in carrot juice (Brix 6%) at 37°C for 14 h, and stored at 5°C. YSP was prepared from whole soy milk containing soybean fiber, okara, as the main material, and fermented carrot juice containing P. pentosaceus IDS885m4, sugar, agar, flavor, citric acid and guar gum were used as ingredients. After the addition of B. animalis subsp. lactis OPB-1, the mixture was stored in a paper cup and refrigerated to 5°C. The nutrient content of YSP was as follows: energy 69 kcal/100 g, protein 3.3 g/100 g, lipid 2.1 g/100 g, carbohydrate 8.5 g/100 g, dietary fiber 1.5 g/100 g, Na 37 mg/100 g. The number of OPB-1 was 2.5 × 10^{8} CFU/g (plate count method) and 4.0 × 10^{11} copies/g (quantitative real-time PCR). YSP also contained 7.9 × 10^{7} CFU/g of P. pentosaceus IDS885m4. The number of bacteria in YSP was stable at 5°C, 4 weeks after production.

Subjects and study design Twelve healthy subjects, 5 women and 7 men, aged 25–60 years (mean age, 42.7 years) were selected for this study. The inclusion criteria were: no history of digestive pathology and no use of medications affecting the intestinal microbiota, such as antibiotics, antacids or laxative drug treatments. The subjects did not have a history of soy allergy or intolerance, nor did they exhibit other symptoms during the study. The study was divided into 3 periods: a wash out period (2 weeks), YSP administration period (3 weeks) and post-administration period (1 week). The amount of YSP administered was 100 g/day. Throughout the study period, the subjects were asked to avoid fermented milk, oligosaccharides, kimchi, fermented soybean (natto) and any food or antibiotic that might influence the fecal microbiota, other than that used in the study. Besides these, there were no specific dietary restrictions. Faecal samples were collected from each subject before administration, at weeks 2 and 3 during the administration period, and at week 1 after administration. The study was approved by the local Ethic Committee of Emilio Moriguchi Clinic, and was conducted based on the principles of the World Medical Association Declaration of Helsinki (1964), under the management and guidance of a physician. Informed consent was obtained from all subjects prior to the start of the study.

Examination of fecal samples and genus- and species-specific PCR The numbers of Bifidobacterium, Bacteroides, and total anaerobes were determined within 24 h of fecal sample collection, according to the method of Mitsuoka (1980). Fecal samples were weighed and serial 10-fold dilutions (from 10^{-1} to 10^{8}) were prepared in solution [Salt sol. I (0.78% K2HPO4) 37.5 mL, Salt sol. II (0.47% KH2PO4, 1.18% NaCl, 1.2% (NH4)2SO4, 0.12% CaCl2, 0.25% MgSO4·7H2O) 37.5 mL, 0.1% Resazurin 1 mL, L-Cysteine HCl·H2O 0.5 g, 25% L-Ascorbic acid 2 mL, 8% Na2CO3 50 mL, Agar 0.5 g, Distilled water 860 mL] and plated on the appropriate agar media. Bifidobacterium and Bacteroides were enumerated on BS agar (Mitsuoka, 1980) and BBE agar (Becton Dickinson, Sparks, MD), respectively. BL agar (Eiken Chemical, Tokyo, Japan) and CDC agar (Becton Dickinson) were used for enumeration of total anaerobes. The plates were incubated at 35°C for 3–5 days, in an anaerobic jar with an atmosphere of oxygen-free CO2 using AnaeroPack-Anaero (Mitsubishi Gas Chemical, Tokyo, Japan). Bacterial densities were expressed as log CFU/g wet weight of feces. After incubation, 10 colonies from each BS agar plate were applied to genus- and species-specific multiplex-PCR to distinguish B. animalis subsp. lactis from other Bifidobacterium, according to the procedure of Ventura et al. (2001). The number of B. animalis subsp. lactis was calculated from B. animalis subsp. lactis-specific PCR and the number of Bifidobacterium on BS agar plates. The primers used in this study are shown in Table 1. SapphireAmp Fast PCR Master Mix (Takara Bio, Shiga, Japan) was used for DNA extraction from colonies. Amplified products were analyzed by 1.5% agarose gel electrophoresis in TAE buffer and visualized by staining with EzVISION (Amresco, Cleveland, OH) and photographed under UV light. The amplification fragment with the primer pair Bflact2/Bflact5 could be obtained only with DNA derived from B. animalis subsp. lactis OPB-1 and B. animalis subsp. lactis JCM10602, whereas no PCR product could not be detected with those primers for any other Bifidobacterium, such as Bifidobacterium longum subsp. infantis JCM1222, B. longum subsp. longum JCM1217, Bifidobacterium adolescentis JCM1275, Bifidobacterium breve JCM1192, and Bifidobacterium bifidum JCM1255. In contrast, about 1400
bp amplicons with the primer set Lm3/P0 were detected from these *Bifidobacterium* strains without *B. animalis* subsp. *lactis*.

**Quantitative real-time PCR**  Real-time PCR was used to quantify bifidobacteria and *B. animalis* subsp. *lactis* in fecal samples. Fecal material (0.1 g) was suspended in 0.9 mL of phosphate buffered saline, and 200 μL of the suspension was used for DNA extraction with Magtration System 12GC (Precision System Science, Chiba, Japan), following the manufacturer's instructions. Quantitative real-time PCR was performed using Rotor-Gene Q (Qiagen, Hilden, Germany). The reaction mixture (25 μL) was composed of 12.5 μL of SYBR:Premix Ex Taq (Takara Bio, Shiga, Japan), 0.2 μL of DNA extraction buffer, and 0.2 μM of each specific primer (Table 1). The amplification program used was as follows: preheating 95°C for 30 sec, 35 cycles of denaturation at 94°C for 5 sec, annealing at 64°C for 20 sec (for *Bifidobacterium* genus specific primer, Lm26/Bif228) or 58°C for 45 sec (for *B. animalis* subsp. *lactis* specific primer, Bflact2/Bflact5) and extension at 72°C for 90 sec. The quantities of target copies contained in an unknown sample were determined from the standard curve. The standard curve was constructed comparing the cycle threshold of each sample with the number of copies. *B. longum* JCM1217 and *B. animalis* subsp. *lactis* OPB-1 were used as standard DNA. The primer set (Lm26/Bif228) was designed for *Bifidobacterium* genus from the intestinal tract. Therefore, a difference of 2 bases is recognized between the DNA sequence of forward primer (Lm26) and the 16S rDNA sequence of *B. animalis* subsp. *lactis*. However, amplicons were obtained with 3.9% of the copy number of *B. animalis* subsp. *lactis* OPB-1, when PCR was performed using Lm26/Bif228. Therefore, a correction factor “0.961” was used for calculation of total *Bifidobacterium* as follows: Total *Bifidobacterium* (copy/mg DNA) = *Bifidobacterium* genus (using Lm26/Bif228, copy/mg DNA) + 0.961· *B. animalis* subsp. *lactis* (using Bflact2/Bflact5, copy/mg DNA).

**Table 1.** Primers used in this study.

<table>
<thead>
<tr>
<th>Primer</th>
<th>Sequence 5’ → 3’</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>For identification of colonies on BS agar plates</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Bifidobacterium</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lm3</td>
<td>CGGGTGCTNCCACTTCTATG</td>
<td>Kaufman et al. (1997)</td>
</tr>
<tr>
<td>P0</td>
<td>GAGAGTTTGATCCTGGCTCAG</td>
<td>DiCello. and Fani (1996)</td>
</tr>
<tr>
<td><strong>B. animalis subsp. lactis</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bflact2</td>
<td>GTGGAGACACCGGGTTCCC</td>
<td>Ventura et al. (2001)</td>
</tr>
<tr>
<td>Bflact5</td>
<td>CACACCACACAAATCCAAATAC</td>
<td>Ventura et al. (2001)</td>
</tr>
<tr>
<td><strong>For quantitative PCR</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Bifidobacterium</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lm26</td>
<td>GATTCCTGCTCAGGATGAACGC</td>
<td>Kaufman et al. (1997)</td>
</tr>
<tr>
<td>Bif228</td>
<td>CTGATAGGACGCGACCACCAT</td>
<td>Marteu et al. (2001)</td>
</tr>
<tr>
<td><strong>B. animalis subsp. lactis</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bflact2</td>
<td>GTGGAGACACCGGGTTCCC</td>
<td>Ventura et al. (2001)</td>
</tr>
<tr>
<td>Bflact5</td>
<td>CACACCACACAAATCCAAATAC</td>
<td>Ventura et al. (2001)</td>
</tr>
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</table>

**T-RFLP**  Terminal restriction fragment length polymorphism (T-RFLP) was performed using the procedure of Nagashima et al. (2006) at TechnoSurga Laboratory (Shizuoka, Japan). In brief, frozen fecal specimens were suspended in GTC buffer (100 mM Tris-HCl buffer, pH 9.0, 40 mM Tris-EDTA buffer, pH 8.0, 4M guanidine thiocyanate). Fecal solids in the suspension were broken down using a FastPrep FP100A Instrument (MP Biomedicals, CA) with zirconia beads at 5 m/sec for 2 min. DNA was extracted from a 200 µL suspension using an automatic nucleic acid extractor (Precision System Science, Chiba, Japan). MagDEA DNA200(GC) (Precision Science) was used as the reagent for the automatic nucleic acid extraction. The PCR primer for 516F labeling was switched from HEX to FAM. PCR products were purified using a MultiScreen PCRμ96 plate (Millipore, MA). The resulting 16S rDNA amplicons were treated with 10U of BsaI (New England BioLabs, MA) for 3 h. Fragment analysis was performed with a ABI PRISM 3130xl DNA Sequencer (Applied Biosystems, Carlsbad, CA) using the DNA analysis software Gene Mapper (Applied Biosystems). MapMarkerR, 50  − 1000 bp, X-Rhodamine conjugate (BioVentures, Murfreesboro, TN) was used as the size standard marker. Bacterial flora was compared by hierarchical cluster analysis (GeneMaths; Applied Maths, Belgium) of the ratio of the peak area to the total area of each OTU.
Bacterial numbers were converted to log colony forming units (CFU)/g wet weight and expressed as means ± SD (standard deviation). For the quantitative PCR, results were converted to log copy numbers/mg DNA extract. Results obtained at various time points were compared using a two-tailed paired t-test. Calculations were performed using SPSS 19.0 software (SPSS Inc, Chicago, IL) and p < 0.05 was regarded as significant.

Results

Quantification of total Bifidobacterium and B. animalis subsp. lactis in feces

Before administration, the number and number of total Bifidobacterium and B. animalis subsp. lactis were not detected by the plate count method, and the number and number of total Bifidobacterium and B. animalis subsp. lactis decreased, as assessed by quantitative real-time PCR. The number of cultivable Bifidobacterium combined with genus- and species-specific PCR and quantitative real-time PCR, at weeks 2 and 3 of the administration period was 2.4 × 10^10 CFU/day by the plate count method and 4.2 × 10^13 copies/day by quantitative real-time PCR. On the other hand, the number of excreted B. animalis subsp. lactis OPB-1 was estimated as 3.2 × 10^11 CFU/day and 7.9 × 10^11 CFU/day by the plate count method combined with species-specific PCR and 7.9 × 10^14 copies/day and 9.7 × 10^14 copies/day by quantitative real-time PCR, at weeks 2 and 3 of the administration period, respectively (Fig. 1).

Effect on fecal microbiota

The number of total Bifidobacterium, Bacteroides and total anaerobic bacteria were analyzed before, during the 2 and 3 weeks of administration and one week post-administration of YSP. Relative abundance of Bifidobacterium and Bacteroides in total anaerobic bacteria is shown in Fig. 2. In 10 cases of 12 subjects, relative abundance of Bifidobacterium increased and that of Bacteroides decreased in 11 of 12 subjects, at week 3 of YSP ingestion. Relative abundance of Bifidobacterium increased from 16.9 ± 21.8% to 21.8 ± 16.0% and 34.1 ± 20.4%, while relative abundance of Bacteroides decreased from 54.0 ± 21.4% to 33.8 ± 23.0% and 33.6 ± 17.9%, at weeks 2 and 3 of YSP administration.

Table 2. Total fecal Bifidobacterium and B. animalis subsp. lactis OPB-1 concentrations in 12 healthy subjects before, during and after ingestion of YSP.

<table>
<thead>
<tr>
<th></th>
<th>Before administration</th>
<th>Administration period</th>
<th>Post administration</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td>2 weeks</td>
<td>3 weeks</td>
</tr>
<tr>
<td>Plate counts (log_{10} CFU/g feces)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B. animalis subsp. lactis</td>
<td>ND (0/12)</td>
<td>9.1 ± 0.5 (11/12)</td>
<td>9.3 ± 0.6 (11/12)</td>
</tr>
<tr>
<td>Total Bifidobacterium</td>
<td>8.4 ± 2.4 (11/12)</td>
<td>9.9 ± 0.5 (12/12)</td>
<td>10.1 ± 0.6 (12/12)</td>
</tr>
<tr>
<td>Quantitative PCR (copy/mg DNA)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bifidobacterium</td>
<td>11.3 ± 0.7 (12/12)</td>
<td>11.4 ± 0.5 (12/12)</td>
<td>11.6 ± 0.4 (12/12)</td>
</tr>
<tr>
<td>B. animalis subsp. lactis</td>
<td>ND (0/12)</td>
<td>11.9 ± 0.6 (12/12)</td>
<td>12.2 ± 0.5 (12/12)</td>
</tr>
<tr>
<td>Total Bifidobacterium</td>
<td>11.3 ± 0.7 (12/12)</td>
<td>12.1 ± 0.4** (12/12)</td>
<td>12.3 ± 0.4** (12/12)</td>
</tr>
</tbody>
</table>

Mean±SD (standard deviation)

** Significantly higher than before administration (p < 0.01)
1) ND : not detected
2) Frequency of occurrence (no. of subjects yielding the organisms/no. of subjects examined)
3) One week after administration
The numbers of *B. animalis* subsp. *lactis* OPB-1 ingested and excreted daily. The number of excreted *B. animalis* subsp. *lactis* OPB-1 was calculated using fecal bacterial number and average evacuation weight during 2nd and 3rd week of YSP administration.

 administration, respectively. However, one week post-administration, the frequency of *Bifidobacterium* decreased and the frequency of *Bacteroides* increased to initial levels.

Fecal microbiota was analyzed by the T-RFLP method, before and after 3 weeks of YSP ingestion. The ratio of *Bifidobacterium* increased from $18.9 \pm 13.4\%$ to $27.8 \pm 15.8\%$ ($p < 0.05$), and that of *Bacteroides* decreased from $8.2 \pm 5.0\%$ to $4.5 \pm 3.3\%$ ($p < 0.08$). This result is not contradictory to the result of the plate count method. No significant changes were detected in the other bacterial groups (Fig. 3).
Discussion

The present study examined the effect of ingestion of a milk-free yogurt-like soybean product containing *B. animalis* subsp. *lactis* OPB-1 on fecal microbiota by quantifying the number of viable *B. animalis* subsp. *lactis* OPB-1 excreted in the feces. Cultivation, molecular and biochemical approaches were applied to evaluate the recovery of *B. animalis* subsp. *lactis* OPB-1 and fluctuations in composition of gut microbiota in healthy adults before, during and after ingestion of 2.5 × 10^{10} CFU/day of *B. animalis* subsp. *lactis* OPB-1 in YSP for 3 weeks.

The combination of selective cultivation and species-specific PCR techniques allowed detection of cultivable *B. animalis* subsp. *lactis* in the feces of 11 of 12 healthy volunteers after 2 and 3 weeks of YSP ingestion (Table 2). Using quantitative real-time PCR, amplified DNA fragments corresponding to *B. animalis* subsp. *lactis* were detected in all subjects during YSP administration. Before administration of YSP, these amplified DNA fragments were not detected in all subjects. Therefore, the amplified DNA fragments corresponding to *B. animalis* subsp. *lactis* originating from the *B. animalis* subsp. *lactis* OPB-1 in YSP. Furthermore, the number of excreted *B. animalis* subsp. *lactis* OPB-1 per day was estimated to be more than 10 times the intake number (Fig. 3). These results suggest that *B. animalis* subsp. *lactis* OPB-1 can survive passage through the gastrointestinal tract and proliferate in the digestive tract. Ishizuka *et al.* (2010) reported that *B. animalis* subsp. *lactis* OPB-1 and fluctuations in composition of gut microbiota in healthy adults before, during and after ingestion of 2.5 × 10^{10} CFU/day of *B. animalis* subsp. *lactis* OPB-1 in YSP for 3 weeks.

The number of viable *B. animalis* subsp. *lactis* OPB-1 and fluctuations in composition of gut microbiota in healthy adults before, during and after ingestion of 2.5 × 10^{10} CFU/day of *B. animalis* subsp. *lactis* OPB-1 in YSP for 3 weeks.

The percentage of *Bifidobacterium* in the fecal microbiota and the number of total *Bifidobacterium* in feces increased during YSP administration. However, the number of *Bifidobacterium*, except for *B. animalis* subsp. *lactis*, did not show a remarkable change, as assessed using the plate count method combined with species-specific PCR and genus-specific quantitative real-time PCR, before, during and post administration (Table 2). The relative abundance of *Bifidobacterium*, except *B. animalis* subsp. *lactis*, did not change by the T-RFLP method, before and at 3 weeks of YSP administration (data not shown). These results suggest that increments in the number and relative abundance of total *Bifidobacterium* were brought about by an increase of *B. animalis* subsp. *lactis* OPB-1. It was reported that ingestion of 2−6 g of soybean oligosaccharides increased *Bifidobacterium* in the human intestine (Tashiro, 2006). YSP contained 0.4 g/100 g of stachyose and 0.08 g/100 g of raffinose, similar to commercial soymilk. In this study, the number of total *Bifidobacterium* increased by the ingestion of YSP; however, the number of *Bifidobacterium*, except *B. animalis* subsp. *lactis* OPB-1, did not remarkably change. The oligosaccharide content in YSP may not be sufficient to increase the population of indigenous *Bifidobacterium*. In addition, *B. animalis* subsp. *lactis* OPB-1 might compete with indigenous *Bifidobacterium* for utilization of oligosaccharides in the gastrointestinal tract. Kumemura *et al.* (2002) reported that administration of a cultured vegetable drink containing *P. pentosaceus* IDS885 altered the composition of fecal microflora in a beneficial manner for human health. *P. pentosaceus* IDS885 contained in YSP might affect the change in composition of fecal microbiota observed in this study.

One week post-administration, *B. animalis* subsp. *lactis* OPB-1 was not detected by the plate count method, and the number and detection rate of *B. animalis* subsp. *lactis* OPB-1 in feces tended to decrease, as assessed by quantitative real-time PCR (Table 1). Therefore, this strain might not have a strong adhesive ability toward intestinal mucosal cells. It was reported that some human bifidobacterial strains show the ability to adhere to intestinal cells in vitro (Bernet *et al.*, 1993, Perez *et al.*, 1998). However, few reports show that ingested bifidobacteria colonize the human gut continuously for a long term (Bezkorovainy, 2001). Kullen *et al.* (1997) fed a unique *Bifidobacterium* strain to human volunteers and then examined the fecal bifidobacterial flora. While the feeding continued, total bifidobacterial excretion increased; however, this strain disappeared from the feces after feeding was discontinued. From these results, they described that exogenous bifidobacteria can not colonize the gastrointestinal tract to a significant extent. Similar results were obtained by
Survival of *B. animalis* subsp. *lactis* OPB-1

Bouhnic *et al.* (1992), and they concluded that administered *Bifidobacterium* spp. do not colonize the human colon. A pulsed-field gel electrophoresis (PFGE) analysis of fecal *B. longum* isolates of 12 human subjects revealed that each subject harbored strains of unique clones and most of them had persisted in the host intestine for over 68 weeks (Kohara *et al.*, 2006). In addition, PFGE profiles of these isolates were different from that of any *B. longum* commercially available. These results suggest that indigenous *Bifidobacterium* strains colonize all individuals’ intestines. These findings lend credibility to the following notion: it may not be so important that the probiotic strains are isolated from human intestine and have strong adhesive ability to intestinal epithelium *in vitro*. If one assumes that increasing fecal bifidobacteria is the primary requisite for achieving probiotic effects, it seems important that we ingest a probiotic strain that can survive and proliferate continuously in the digestive tract.

In this study, we demonstrated that *B. animalis* subsp. *lactis* OPB-1 in a soybean product free of milk components can survive and proliferate in the gastrointestinal tract of healthy adults. Moreover, ingestion altered the composition of fecal microbiota in a manner that exerted a beneficial effect on human health. Yogurt-like soybean product that contains *B. animalis* subsp. *lactis* OPB-1 is a promising probiotic source for people wishing/needing to avoid milk components, and further research is expected in the future.

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**References**


