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ARTICLE

Adhesive Properties of *Lactobacillus brevis* FSB-1 *In Vivo*

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Abstract

This study was conducted to evaluate the *in vivo* gastrointestinal survival and adhesive properties of orally administered *Lactobacillus brevis* FSB-1. ELISA conducted using polyclonal antibodies specific for *L. brevis* FSB-1 was able to detect the organism in feces; therefore, we used ELISA to determine the concentration of lactic acid bacteria in feces collected from Wister rats that had been administered 10^{10} cells/rat/d orally for 20 d. The mean recovery of *L. brevis* FSB-1 was approximately $10^{7.22}$ cells/g of wet feces during the oral administration period, and $10^{7.50}$ and $10^{7.46}$ at 8 and 10 d after the end of oral administration, respectively. These results indicate that *L. brevis* FSB-1 was able to survive in the gastrointestinal tract of rats, and that it had a high adhesive property in rat colons.

Key words: *Lactobacillus brevis* FSB-1, adhesive property, polyclonal antibody, ELISA, *in vivo*

Introduction

Lactobacillus and *Bifidobacterium* species are used in the production of traditional fermented foods such as *kimchi*, soybean paste and various dairy products, as well as in medicine and as feed additives (Jung, 1997; Jung and Kang, 1997; Kim, 1994). Lactic acid bacteria (LAB) are considered to be beneficial microorganisms that reduce lactose intolerance, prevent constipation and diarrhea, inhibit the growth of pathogenic bacteria, reduce serum cholesterol, and exert antitumor and immunopotentiating activities (Collins *et al*., 1998; Gill, 2003; Goldin, 1998; Klaenhammer and Kullen, 1999; Ouwehand *et al*., 1999a; Salminen *et al*., 1999; Sherwood and Gorbach, 2000). LAB must remain stable in the human gastrointestinal tract to exert these physiological effects, as well as to ensure their long-term survival (Coconnier *et al*., 1992). In addition, attachment of LAB to human colonic mucosa may prolong their probiotic effects. Therefore, the *in vitro* adhesion ability is considered to be an important selection criterion for potential probiotic strains (Ouwehand *et al*., 1999b, 2001).

Lactobacillus brevis has been isolated from milk, cheese, human feces, and the mouth and the gut of humans. In addition, strains of this species are considered to be GRAS (Generally Recognized As Safe) (Elina *et al*., 2003; Kandler and Weiss, 1986; Ouwehand *et al*., 2001). Recently, Elina *et al*. (2003) reported that *Lactobacillus brevis* ATCC 14869T and ATCC 8287 showed a high binding affinity for human Caco-2 and Intestine 407 cells *in vitro*. Furthermore, *L. brevis* PEL1 has been found to have a high binding affinity to human colonic mucin (Ouwehand *et al*., 2001). Additionally Hynonen *et al*. (2002) reported that the S-layer protein of *L. brevis* ATCC 8287 affected the binding affinity of human Caco-2 and Intestine 407 cells, the endothelial cell line, EA-hy 926, and the urinary bladder cell line, T 24, and that it also immobilized fibronectin. In addition the S-layer protein of *L. brevis* is known to contribute to adhesion properties in gut epithelial cells (Kahala *et al*., 1997).

L. brevis has resistance against low pH, bile salt and pancreatic juice flow (Elina *et al*., 2003); therefore, several studies have shown that it has the potential for use as a probiotic (Elina *et al*., 2003; Kishi *et al*., 1996; Maassen *et al*., 2000; Playfair, 1987). However, the ability of *Lactobacillus* and *Bifidobacterium* to survive in the gastrointestinal tract varies considerably among species and strains. Therefore, it is important to assess the ability of specific bacterial strains to survive in the gastrointestinal tract. One factor that is important in determining the ability of these organisms to survive is their adhesive properties (Yuki *et al*., 1999).

In a previous study, we found that *Lactobacillus*

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brevis FSB-1 had the ability to bind to human colonic mucosa *in vitro*, as well as to exert immunopotentiating properties (Kim *et al*., 2004a, 2004b). Therefore, we conducted this *in vivo* study using Wister rats to determine the ability of *L. brevis* FSB-1 to adhere to the gut mucosa and to survive in the gastrointestinal tract. In addition we developed a simple and accurate method of isolating and identifying *L. brevis* FSB-1 cells in feces collected from Wister rats using ELISA with specific polyclonal antibodies against *L. brevis* FSB-1 that were produced in rabbits.

Materials and Methods

Microorganisms and animals

The *Lactobacillus brevis* FSB-1 used in this study had been determined to have the greatest adhesive affinity for rat colonic mucin (RCM) during screening conducted as part of a previous study (Kim *et al*., 2004a). The experimental animals used in this study included Wister rats (male, 10 wk, Samtaco Co. Ltd., Korea) and New Zealand white rabbits (male, weight 270 g, Biogenomics Co. Ltd., Korea). All animals were acclimated in a cage with a controlled atmosphere (temperature 24±1°C; relative humidity 55±1%) for 24-48 h prior to the experiment, during which time feed (Samyang Co. Ltd., Korea) and water were provided *ad libitum*.

Preparation of polyclonal antibodies

Activated *L. brevis* FSB-1 was cultivated in 300 mL of MRS broth (1%, v/v) at 37°C for 48 h. The cells were then harvested by centrifugation at 4°C and 6,000 rpm for 20 min, after which they were washed 3 times with 10 mM phosphate buffered saline (PBS, pH 7.2). Next, the cells were resuspended in 10 mM PBS (pH 7.2), after which they were treated with heat (100°C) for 10 min, and then adjusted to a concentration of 2×10^8 cells/mL in PBS (pH 7.2). Five hundred μL of the *L. brevis* FSB-1 cell suspension (2×10^8 cells/mL) and 500 μL of Freund's complete adjuvant were then homogenized to obtain a stable emulsion of water in oil. Next, 1 mL of the cell emulsion (10^8 cells/mL) was injected into the muscle in the hips at the quarter point of a rabbit. Two weeks later, the rabbit was given a booster shot of 10^8 cells/mL. After one week, the serum containing IgG (polyclonal antibodies) was obtained from the rabbit (Bouh and Mittal, 1999; Hay and Westwood, 2002a; Kim and Slauch, 1999; Raamsdonk *et al*., 1995).

Enzyme linked immunosorbent assay (ELISA)

ELISA was conducted using the method described by Voller *et al.* (1976). Briefly, *L. brevis* FSB-1 cells were washed 3 times with 10 mM PBS (pH 7.2) and then resuspended in 0.05 M carbonate buffer (0.05 M Na_2CO_3 and $NaHCO_3$, pH 9.6) at a concentration that gave an absorbance of 1.0 at 660 nm. One hundred μL of the *L. brevis* FSB-1 cell suspension were then poured into a microtiter plate (Maxisorp, Nunc, Denmark) and incubated overnight at 4°C. Various bacteria cells (Table 1) were also poured into a microtiter plate and incubated under the same conditions to evaluate the dose response relationship. The plates were then washed 3 times with PBST (PBS containing 0.05% Tween 20, pH 7.2), after which they were incubated with 120 μL of blocking buffer (1% BSA/0.05 M carbonate buffer, pH 9.6) at 37°C for 1 h. Next, the plate was washed 3 times with PBST, after which 90 μL of rabbit-anti-*L. brevis* FSB-1 IgG polyclonal antibodies (×25,000 dilution with blocking buffer) were added to the plates. The plates were then incubated at 37°C for 1.5 h. Next, the plates were washed 3 times with PBST, and then 100 μL of peroxidase-goat anti-rabbit IgG (H+L) (×12,000 dilution with blocking buffer, Zymed Lab. Inc., USA) were added. The plates were incubated at 37°C for 1.5 h, washed 7 times with PBST, and then refilled with 60 μL of the 3,3',5,5'-tetramethylbenzydine (TMB) liquid substrate system (Sigma Chemical Co., USA). The reaction was then stopped by the addition of 60 μL of 1 M H_2SO_4 (reagent first grade), after which the absorbance at 450 nm was measured using a Microtiter plate reader (Molecular Devices, USA).

Intake of *L. brevis* FSB-1 cells by Wister rats

Growth and pretreatment of *L. brevis* FSB-1

The activated *L. brevis* FSB-1 were cultivated in 300 mL of MRS broth (inoculum size, 1%, v/v) at 37°C for 48 h. The cells were then harvested by centrifugation at 4°C and 6,000 rpm for 20 min, after which they were washed 3 times with 0.9% saline. Next, the cells were then adjusted to a concentration of 10^{10} cells/mL in 0.9% saline.

Determination of the survival ability and adhesive properties of *L. brevis* FSB-1 in rat colons

This experiment was conducted using 6 Wister rats (male, 3 wk old) that were divided into an experimental and a control group. Each group of animals was kept in a cage with a controlled atmosphere (temperature 24±1°C; relative humidity 55±1%) for the entire experimental

period, during which time feed (Samyang Co. Ltd., Korea) and water were provided *ad libitium*.

During the study, rats in the experimental group were orally administered 1 mL of the suspension of *L. brevis* FSB-1 (10^{10} cells/mL, 0.9% saline) described above and rats in the control group were orally administered 1 mL of 0.9% saline once a day (in the afternoon) for 20 consecutive days. Fecal samples were obtained from each group within 24 h of the last oral administration (oral administration period; 1-20 d) and after the end of oral administration (21-42 d beyond 24 h the last oral administration).

Determination of the transit time of *L. brevis* FSB-1 in the gastrointestinal tract

This experiment was conducted using Wister rats 6 (male, 3 wk old) that were divided into an experimental and a control groups. Each groups of animals was kept in a cage with a controlled atmosphere (temperature 24±1 °C; relative humidity 55±1%) for the entire experimental period, during which time feed (Samyang Co. Ltd., Korea) and water were provided *ad libitium.*

During the study, rats in the experimental group were orally administered 1 mL of the suspension of *L. brevis* FSB-1 (10^{10} cells/mL, 0.9% saline) described above and rats in the control group were orally administered 1 mL of 0.9% saline once a day (in the afternoon) for 10 consecutive days. Fecal samples were then collected at 0, 2, 4, 6, 8, 10, 12, and 24 h after the last treatment was administered.

Determination of the concentration of *L. brevis* FSB-1 in Wister rat feces

Fresh Wister rat feces (1 g) were suspended in 9 mL of 10 mM PBS (pH 7.2) and the residue (unessential materials) was then removed by centrifugation at 4°C and 1,500 rpm for 2 min. The supernatant was then removed and centrifuged at 4°C and 6,000 rpm for 20 min to collect the pure microflora in the feces. The microflora were then washed 3 times with 10 mM PBS (pH 7.2), after which they were resuspended in 0.05 M carbonate buffer (pH 9.6). The concentration of *L. brevis* FSB-1 in the wet feces was then determined based on the absorbance of samples following ELISA using polyclonal antibodies.

Statistical analysis

Experimental data were expressed as the mean±SD. The treatment and control groups were compared using a student's *t*-test and then evaluated to determine if the values differed using $p<0.05$, $p<0.01$ or $p<0.001$ to indicate statistical significance.

Results

Susceptibility of polyclonal antibodies to *L. brevis* FSB-1

To determine the specificity for the *L. brevis* FSB-1 antigen and the detection limit of the polyclonal antibodies generated here, we evaluated the relationship between the concentration of *L. brevis* cells and the polyclonal antibodies produced using the ELISA method (Fig. 1). The results revealed that the absorbance increased proportionally as the number of cells (*L. brevis* FSB-1) increased. Specifically, as the number of cells increased from $10^{6.04}$ to $10^{7.85}$ cells/mL, the absorbance increased from 0.12 to 1.72. However, concentrations of less than 10^6 and greater than 10^8 cells/mL could not be evaluated using the ELISA method with the polyclonal antibodies developed here.

To determine the specificity of the polyclonal antibodies against *L. brevis* FSB-1 produced for this study, we measured the cross-reactivity among *L. brevis* FSB-1 and 7 *Lactobacillus* species, as well as the LAB, *Bifidobacterium bifidum* KCTC 3357*, Lactococcus lactis, Leuconostoc mesenteroides* ssp. *mesenteroide*s ATCC 8293*, Pediococcus pentosaceus* KFRI 833, and *Enterococcus faecalis* KCTC 2011*,* and the pathogenic bacteria, *Escherichia coli* MC 106 and *Staphylococcus aureus*. The results revealed that a high level of cross-reactivity existed between the antibodies and *L. plantarum* ATCC 14917, *P. pentosaceus* KFRI 833, *L. helveticus* ATCC 8018 and *L.*

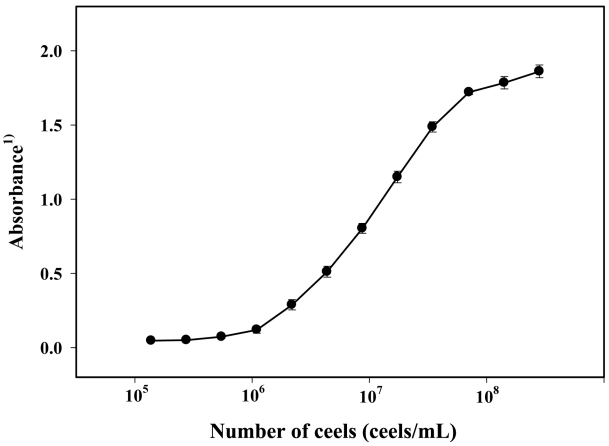

Fig. 1. Detection of *L. brevis* FSB-1 by ELISA using specific polyclonal antibodies. [1)]Absorbance was measured by ELISA at 450 nm and was expressed as the mean±SD of triplicate tests.

Table 1. Specificity of polyclonal antibodies against *L. brevis* FSB-1 produced using rabbits

Bacteria	Relative ELISA titer[1)] (mean±SD)
Lactobacillus brevis FSB-1	1.00±0.00
Lactobacillus brevis ATCC 14869T	0.70±0.01
Lactobacillus acidophilus IFO 3025	0.31±0.00
Lactobacillus casei KCTC 3109	0.30±0.02
Lactobacillus fermentum ATCC 14931	0.15±0.00
Lactobacillus helveticus ATCC 8018	0.74±0.05
Lactobacillus plantarum ATCC 14917	0.49±0.00
Bifidobacterium bifidum KCTC 3357	0.04±0.00
Lactococcus lactis	0.09±0.03
Leuconostoc mesenteroides ssp. *mesenteroide*s ATCC 8293	0.05±0.00
Pediococcus pentosaceus KFRI 833	0.59±0.03
Streptococcus thermophilus ATCC 14485	0.33±0.02
Enterococcus faecalis KCTC 2011	0.21±0.01
Escherichia coli MC 106	0.04±0.00
Staphylococcus aureus	0.07±0.00

[1)]The relative ELISA titer was determined based on the absorbance at 450 nm following ELISA and was expressed as the mean±SD of triplicate tests.

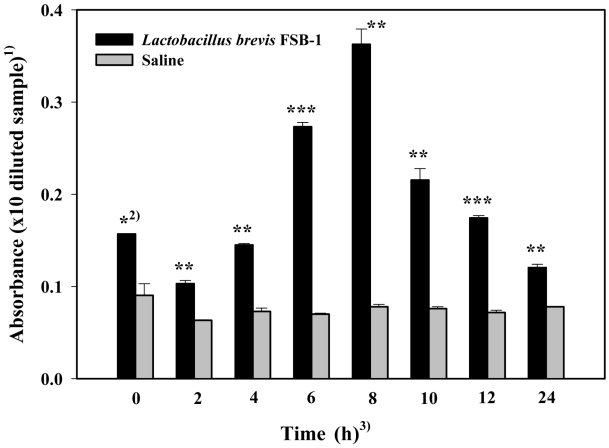

Fig. 2. Determination of the concentration of *L. brevis* FSB-1 in rat fecal samples based on the passage of the time from the last oral administration of *L. brevis* FSB-1. [1)]Absorbance was measured by ELISA at 450 nm and was expressed as the mean±SD of triplicate tests. [2)]*p<0.05; **p<0.01; ***p<0.001, when the control (only saline) and experimental groups were compared. [3)]Time (h), Rat fecal samples were collected on the tenth day after daily oral administration of 10^{10} cells/mL of *L. brevis* FSB-1. 0, 24 h after 9 d treatment; 2, 2 h after 10 d treatment; 4, 4 h after 10 d treatment; 6, 6 h after 10 d treatment; 8, 8 h after 10 d treatment; 10, 10 h after 10 d treatment; 12, 12 h after 10 d treatment; 24, 24 h after 10 d treatment.

brevis ATCC 14869T, and that other strains had lower levels of cross-reactivity (Table 1). The polyclonal antibodies generated here showed especially high levels of cross-reactivity against *L. helveticus* ATCC 8018 and *L. brevis* ATCC 14869T (absorbance of 0.74 and 0.70, respectively, when normalized against the absorbance obtained when *L. brevis* FSB-1 was evaluated).

Survival ability and adhesive properties of *L. brevis* FSB-1 in the gastrointestinal tract

Transit time and survival ability of *L. brevis* FSB-1

As shown in Fig. 2, the absorbance of the experimental group increased from 4 h (0.15±0.00) to 8 h (0.36±0.02), and then gradually decreased at 10 h (0.22±0.01). Conversely, the absorbance of the control group remained constant (0.06±0.00-0.09±0.01) throughout the experiment. In addition, the absorbance of the experimental group was significantly higher than that of the control group at each time point (p<0.05). These findings indicate that *L. brevis* FSB-1 was not detected in the feces of the control group. The results of the experimental group were then used to predict the transit time and estimate the degree of survival of *L. brevis* FSB-1 in the gastrointestinal tract. In other words, we assumed whether *L. brevis* FSB-1 is resistant to the gastric acid, bile acid and pancreatic acid flow in the gastrointestinal tract. Because the absorbance of samples collected from the experimental group was greatest at 8 h, we assumed that this was the transit time of *L. brevis* FSB-1 in the gastrointestinal tract (Fig. 2). At that time, the concentration of *L. brevis* FSB-1 cells in the feces was found to be $10^{7.40}$ cells/g of wet feces.

Determination of the quantity of *L. brevis* FSB-1 in rat fecal samples during the oral administration period

Wister rats (male, 3 wk) were administered either 1 mL of a suspension of *L. brevis* FSB-1 (10^{10} cells/mL) or 1 mL of 0.9% saline once a day (in the afternoon) for 20 consecutive days. The concentrations of *L. brevis* FSB-1 in rat fecal samples constantly collected 24 h after the treatments were then determined. The experimental group was found to have a mean absorbance of 0.20±0.07, while the control group was found to have a mean absorbance of 0.10±0.03 (Fig. 3). The concentration of *L. brevis* FSB-1 was then estimated to be approximately $10^{7.22}$ cells/g of wet feces during the oral administration period based on these values and the data presented in Fig. 1.

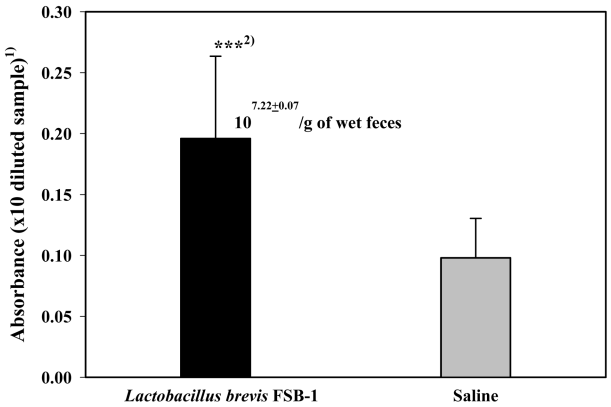

Fig. 3. Determination of the mean recovery of *L. brevis* FSB-1 in rat fecal samples collected daily beginning 24 h after oral administration of *L. brevis* FSB-1 during the administration period. [1)]The absorbance at 450 nm was measured following ELISA and expressed as the mean± SD of triplicate tests. [2)]****p*<0.001 indicating a significant difference between the control (saline only) and experimental groups.

Determination of the quantity of *L. brevis* FSB-1 in rat fecal samples after the end of oral administration

To evaluate the ability of *L. brevis* FSB-1 to survive in and adhere to the gastrointestinal tract, we determined the concentration of *L. brevis* FSB-1 in rat fecal samples collected at the end of the oral administration.

After the end of the oral administration, we found that there was no change in the absorbance of rat fecal samples collected during the first 6 d of the experiment, but that this value was increased at 8 (0.46±0.02) and 10 d (0.42±0.02), after which it decreased again (Fig. 4).

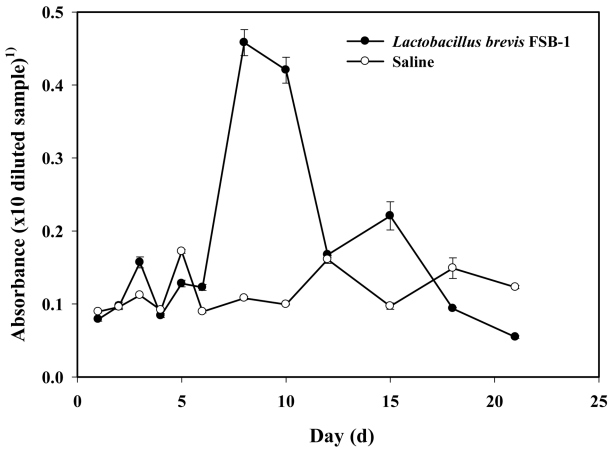

Fig. 4. Determination of the quantity of *L. brevis* FSB-1 in rat fecal samples collected following the oral administration period. [1)]The absorbance at 450 nm was measured following ELISA and was expressed as the mean±SD of triplicate tests.

Based on the results shown in Fig. 1, we assumed that the quantities of *L. brevis* FSB-1 in the rat fecal samples collected at 8 and 10 d were $10^{7.5}$ and $10^{7.46}$ cells/g of wet feces, respectively. Furthermore, the results indicate that *L. brevis* FSB-1 were found in the feces of rats for 11 d following the end point of the oral administration. Additionally, as shown in Fig. 2, the transit time of *L. brevis* FSB-1 was 8 h. Therefore, we assumed that this strain had adhesive properties and was able to survive and proliferate in the colon and then be excreted from the body. In addition, these findings indicated that *L. brevis* FSB-1 was able to persist for at least 11 d following the end of treatment.

Discussion

Polyclonal antibodies can bind to several different antigens. Therefore, polyclonal antibodies that have similar structures to the shape of antigen can show decreased serological specificity as a result of cross-reactivity. Namely, it is likely that the polyclonal antibodies produced in this study were able to react with antigens besides the original antigen (*L. brevis* FSB-1). Nevertheless, the polyclonal antibodies is able to bind to the special antigens, with the consequence that polyclonal antibodies have been used for identification of native antigen or expression of partial base sequence in accomplishment of gene product (Hay and Westwood, 2002b) Based on the above reasons, we evaluated the relationship between the concentration of *L. brevis* cells and the polyclonal antibodies produced using the ELISA method (Fig. 1). And we also determined the specificity of the polyclonal antibodies against *L. brevis* FSB-1 by measuring the cross-reactivity among the various bacterial cells (Table 1). The polyclonal antibodies generated here showed especially high levels of cross-reactivity against *L. helveticus* ATCC 8018 and *L. brevis* ATCC 14869T (Table 1). *L. helveticus* and *L. brevis* are closely related species with similar S-layer protein (43-45 kDa) properties (Jakava-Viljanen *et al*., 2002; Pouwels *et al*., 1998). In addition, Kim *et al*. (2004a) reported that *L. brevis* ATCC 14869T and *L. brevis* FSB-1 shared a similar morphology, biochemical composition and 16S rDNA sequence. The findings of these previous studies are consistent with the cross-reactivity observed in this study. However, because *L. helveticus* is primarily isolated from milk and cheese (Kandler *et al*., 1986), it is not likely that *L. helveticus* would be found in feces. Therefore, the polyclonal antibodies against *L. brevis* FSB-1 used in this

study were considered to effectively detect *L. brevis* FSB-1 in feces. In addition, the above results (Fig. 1, Table 1) indicate that the detection of bacteria in fecal material by ELISA using polyclonal antibodies could be a useful tool for detecting specific dietary LAB *in vivo*.

Tuleu *et al*. (1999) determined transit time by evaluating the intake (0.5-1 g) of a fibre-free diet based on the size [0.71-1.00 (diameter, mm)] and density (0.9 g/cm^3) of the gastrointestinal tract using Fisher rats (female, 12 wk). The results revealed that the transit time ranged from 3 h to 9 h, and that no feed was found in the colon after 24 h. Although it is difficult to compare the results of that study to those of the present study, based on the time against required for rats to digest the food in the study conducted by Tuleu *et al*., it is likely that the ingested feed and LAB were digested and excreted within 24 h in the present study. Therefore, the presence of *L. brevis* FSB-1 in the rat feces 24 h after treatment was stopped indicates that they have the ability to survive in and adhere to the gastrointestinal tract. In addition, *L. brevis* FSB-1 was administered at a concentration of 10^{10} cells/mL, $10^{7.40}$ cells/g of wet feces were detected 8 h after the initial treatment. Based on this result, we assumed that *L. brevis* FSB-1 was able to survive in the colon, and that this strain could be detected in rat fecal samples because it was resistant to the gastrointestinal tract. Elina *et al*. (2003) reported that *L. brevis* was resistant to low pH, bile acid and pancreatic juice flow, which is similar to the results of the present study.

Nardi *et al*. (1999) reported that *Lactobacillus* species are constantly present in Wister rat feces. However, the mean value of the absorbance in the control group was 0.10±0.03 in the present study, which was below the detection limit for *L. brevis* FSB-1. In addition, the absorbance of the control group was significantly lower than that of the treatment group (p<0.001). Consequently, the absorbance value of the control group was assumed to be the result of nonspecific binding of the polyclonal antibody to organisms other than *L. brevis* FSB-1.

Saxelin *et al*. (1995) reported that when gelatin encapsulated *Lactobacillus* GG strain were ingested by 20 healthy adults, they were present in concentrations that ranged from 1.6×10^8 to 1.2×10^{10} cells/mL for 7 consecutive days. The results of that study also indicated that the organism was not detected in feces from the intake group that was administered 1.6×10^8 *Lactobacillus* GG strain cells/mL, but that it was detected in concentrations of 2.0×10^6 cells/g in feces collected from an intake group that was administered 1.2×10^{10} cells/mL during 3 to 7 d of the intake period. Even though the study conducted by Saxelin *et al*. utilized encapsulated *Lactobacillus* GG strain and our study utilized orally administered *L. brevis* FSB-1 in 0.9% saline, *L. brevis* FSB-1 was still detected in a concentration of 10^7 cells/g of wet feces during the oral administration period of the study. Therefore, the results of this study indicate that *L. brevis* FSB-1 has greater survival ability than *Lactobacillus* GG strain in the gastrointestinal tract.

Huang *et al*. (2003) administered *Propionibacterium jensenii* 702 orally to Wister rats at a concentration of 10^{10} cells/mL once a day for 81 consecutive days and then determined the quantity of *P. jensenii* 702 in their feces. The results of that study revealed that *P. jensenii* 702 were present at a concentration of 10^8 cells/g of feces following treatment for 36 d. In addition, *P. jensenii* 702 were detected at a concentration of 10^8-10^9 cells/g of feces after treatment for 81 d. Based on these results, Huang *et al*. (2003) reported that *P. jensenii* 702 were able to survive in the gastrointestinal tract and proposed that the evaluation of new probiotics must be conducted using a treatment dose that is greater than 10^7 cells/d. In the present study, *L. brevis* FSB-1 was orally administered to Wister rats at a concentration of 10^{10} cells/d for 20 consecutive days and no adverse effects such as strange behavior, diarrhea or vomiting were observed during the treatment period. This finding indicates that high concentrations of *L. brevis* FSB-1 were not harmful effect to Wister rats during the oral administration period (20 d).

Yuki *et al*. (1999) evaluated the effects of ingesting fermented milk containing *Lactobacillus casei* Shirota at a concentration of 10^{10} cells/mL in 8 healthy male humans for 3 consecutive days and found that *L. casei* Shirota was detected at a concentration of 10^7 cells/g of feces in samples collected on the 4th day of treatment.

Huang *et al*. (2003) reported that at least 10^7 cells/g of feces must be present in the colon for valuable physiological effects of probiotic bacteria to occur. However, Lee and Salminen (1995) reported that only 10^5 cells/g of feces were required for probiotic bacteria to exert a physiological effect on the host. Furthermore, Plant and Conway (2002) reported that lactobacilli with adhesive properties were able to survive better in the gastrointestinal tract than lactobacilli that did not have adhesive properties. Based on the results of these prior reports and this study, we concluded that *L. brevis* FSB-1 was suitable for use as a probiotic because greater than 10^7 cells/g of wet feces were detected during the oral administration period, as well as for 11 d after treatment was stopped. However,

constant intake of *L. brevis* FSB-1 will be required for its probiotic effects to be maintained and to enable it to persist in the colon.

Massen *et al*. (2000) reported that the immunological activity of TNF-α, IL-2 and IL-1β were induced by oral administration of *Lactobacillus brevis* ML 12 at a concentration of 10^{10} cells/mL. In addition, we previously reported that *L. brevis* FSB-1 had immunopotentiating properties such as the ability to induce bone marrow proliferation, macrophage activity, and the activity of the spleen and its complements (Kim *et al*., 2004b). Consequently, *L. brevis* FSB-1 have the ability to survive in the gut, as well as the ability to adhere to the gastrointestinal tract while exerting positive physiological effects. Therefore, *L. brevis* FSB-1 may have the potential for use as a probiotic organism.

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