Inulin-Type Fructans Stimulated the Growth of Exogenously Administered Lactobacillus plantarum No. 14 in the Mouse Gastrointestinal Tract

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This study examined the effects of the inulin-type fructans known as fructo-oligosaccharide (FOS) and inulin on the growth and persistence of Lactobacillus plantarum No. 14 (LP14) in the gut. Cultivation in medium that contained either FOS or inulin revealed that both fructans supported the growth of LP14. Fecal recovery of viable L. plantarum at 24 and 30 h following administration of LP14 were highest in mice fed inulin, followed by mice fed FOS and the control diet. However, neither of the fructans prolonged the appearance of LP14 in the gut. Cultivation in fecal extract medium prepared from mice fed each of the test diets showed higher growth of LP14 in the mice fed fructans. Histological analysis following fluorescein-stained LP14 administration showed that LP14 was largely localized to the luminal contents. These results suggest that inulin-type fructans support the growth of LP14 in the luminal contents only during their passage through the gut.

Key words: inulin-type fructans; prebiotics; probiotics; Lactobacillus plantarum; mice

Probiotics are defined as viable microbial dietary supplements that have beneficial effects on the health of the host organism.¹ The bacteria genera most often used as probiotics include Lactobacillus and Bifidobacteria. Exogenously administered probiotics survive the passage through the host gastrointestinal tract and influence bacterial ecology and metabolic activity in the distal gut. To date, several health-related effects associated with the intake of probiotics, including improvement of lactose intolerance, immune system function, lipid metabolism, diarrhea, and colon cancer, have been reported.² Sustained effects of probiotics are achieved when probiotic organisms adhere to and colonize the intestinal mucosal surface. However, human and animal studies have revealed that exogenously administered probiotics pass into the feces without adhering.³–⁷ Therefore, administration of probiotics at regular intervals is required to obtain a sustained effect.

Prebiotics are defined as non-digestible food ingredients that benefit the host by selectively stimulating the growth and activity of a limited number of bacteria in the distal gut.³ Prebiotics have also been found to enhance the growth and survival of exogenously administered Bifidobacterium and Lactobacillus strains in the gut.⁷,⁹ Thus a combination of prebiotics and probiotics, generally referred to as synbiotics, can be helpful in promoting the persistence of probiotics in the gut, thereby enhancing their effects in an additive or a synergistic manner.

In our preliminary studies, we found that long-term administration of Lactobacillus plantarum No. 14 (LP14) resulted in a reduction in subcutaneous and abdominal fat accumulation in mice fed a high-fat diet. These findings suggest that this bacterial strain represents a potential candidate probiotic bacterium to prevent diet-induced obesity (unpublished results). In the current study, we aimed to investigate the effects of prebiotics on the growth and persistence of LP14 in the murine gut. Mice were fed a synthetic diet supplemented with inulin-type fructans containing different degrees of polymerization (DP), and fecal recovery of viable L. plantarum was measured using a species-selective culture medium. In vitro culture experiments were also performed to determine the effects of inulin-type fructans on the growth of LP14. Furthermore, in order to determine whether LP14 colonized the mucosal surface of the gut, histological examination of gut tissues was performed following intragastric administration of fluorescein-stained LP14. The prebiotic capacity of inulin-type fructans has been extensively studied.⁰,¹¹ In view of the fact that the fermentative properties of inulin-type fructans are dependent on their molecular structure, particularly their DP,¹²–¹⁵ we compared two different fructans, short-chain fructo-oligosaccharides (FOSs) and inulin, in the present study.

Materials and Methods

Maintenance of L. plantarum No. 14. LP14 was donated by the Research Center of Momoya Co., Ltd. (Saitama, Japan), and was cultured in de Man Rogosa Sharpe (MRS) broth (Becton Dickinson, Franklin Lakes, NJ) at 37°C for 24 h. The bacterial pellet was harvested by centrifugation at 3,000 × g for 10 min and then re-

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Abbreviations: AUC, area under the curve; CFSE, 5-(6)-carboxyfluorescein diacetate succinimidyl ester; CFU, colony-forming unit; DAPI, 4, 6-diamidino 2-phenyl indole; DGGE, denaturing gradient gel electrophoresis; DP, degrees of polymerization; FEM, fecal extract medium; FOS, fructo-oligosaccharide; GLP-1, glucagon like peptide-1; LP14, Lactobacillus plantarum No. 14; LPSM, Lactobacillus plantarum-selective medium; MRS, de Man Rogosa Sharpe; OD, optimal density; PYY, peptide Y; SCFAs, short chain fatty acids; UPGMA, unweighted pair-group method with arithmetic mean.
suspended in MRS broth that contained 20% w/v glycerol, and was stored at −80 °C.

Inulin-type fructans. We used two types of inulin-type fructans with different DP (FOS and inulin). FOS (MeioLio P, donated by Meiji Food Materia, Tokyo) was composed of 1.3% glucose and fructose, 2.5% sucrose, 37.3% 1-kestose, 49.1% nystose, and 9.8% fructosyl-nystose, and the average DP was 3. Inulin (Fuji FF, donated by Fuji Nihon Seito, Tokyo) was composed of 94.8% inulin and the average DP was 16 (ranging from 5 to 30).

Animal handling and diet. All study protocols were pre-approved by the Hokkaido University Research Faculty of Agriculture Animal Use Committee (approval no. 16), and all the animals were maintained in accordance with the guidelines for the care and use of laboratory animals of Hokkaido University.

Female ddY mice (5 weeks old) and female BALB/c mice (5 weeks old) were purchased from Japan SLC (Hamamatsu, Japan) and housed in standard plastic cages in a temperature-controlled (23 ± 2 °C) room under a 12-h light:dark cycle. The mice were allowed free access to food and water throughout the course of the experiments. Following 1 week of acclimatization to a synthetic diet prepared according to AIN-93G, 18 ddY mice were randomly allocated to three groups (six mice per group) and fed a synthetic diet as the control diet for 1 week and then subjected to examination of gut persistence with LP14 and Lactobacilllas johnsonii, as described below. The BALB/c mice were fed a commercial rodent diet (MR stock; Nosan Corporation, Yokohama, Japan) for 2 weeks and then subjected to isolation of L. johnsonii, as described below.

Isolation of L. johnsonii from mouse stomach. In order to isolate a positive control bacterium that would persistently colonize the gut, we isolated a Lactobacillus species from the mouse stomach. The BALB/c mice were anesthetized with diethyl ether and sacrificed by exsanguination from the carotid artery. After a laparotomy, the stomach was excised, washed thoroughly with ice-cold PBS, and homogenized in 50 mM anaerobic phosphate buffer (pH 6.8) containing 0.5 g/l of t-cysteine, 0.5 g/l of Tween 80, and 1 g/l of agar. The samples were then diluted and aliquots were plated onto MRS agar and incubated at 37 °C for 2 d using the AnaeroPack system. Following incubation, a single colony was selected and suspended in MRS broth containing 20% w/v glycerol and stored at −80 °C until required. DNA was then isolated from aliquots of bacterial pellets using Prepman Ultra reagent (Applied Biosystems Japan, Tokyo) following the manufacturer’s instructions. The nearly complete 16S rRNA gene was sequenced using the BigDye Terminator v3.0 cycle sequencing kit (Applied Biosystems Japan) following the manufacturer’s instructions with the universal primers 27F, 5’ AGA GTT TGA TCM TGG CTC AG 3’ and 1492R, 5’ TAC GGY TAC CTT GGY TTG ACC T 3’ (the sequencing primers). The sequence was compared to those in the GenBank database using the BLAST N algorithm. The 1,462-base pair (bp) sequence exhibited 99% similarity to L. johnsonii strain KLDS 1.0731 (GenBank accession no. EU526016).

In order to detect orally administered L. johnsonii specifically in the mouse feces, rifampicin-resistant L. johnsonii was prepared by the methods reported by Frece et al.19 Isolated L. johnsonii was plated on MRS agar containing 100 μg/ml of rifampicin (Wako Pure Chemical Industries, Osaka, Japan) and incubated anaerobically at 37 °C for 2 d using the AnaeroPack system. The antibiotic-resistant bacteria was isolated and used as a positive control in evaluating the persistence of the strain in the mouse gastrointestinal tract, as described below.

Detection of L. plantarum and rifampicin-resistant lactobacilli from mouse feces. After feeding with each test diet for 2 and 28 d, the ddY mice were inoculated intragastrically with 0.2 ml of PBS containing 1 × 10^8 colony-forming units (CFUs) of LP14. Feces were then collected from the individual mice at 0, 6, 24, 30, 48, and 54 h after administration. Recovery of viable L. plantarum was measured using L. plantarum-selective medium (LPSM) 18 comprising 10 g/l of peptone (Nihon Pharmaceutical, Tokyo), 10 g/l of Lab-Lemco powder (Oxoid, Basingstoke, UK), 5 g/l of Bacto yeast extract (Becton Dickinson), 20 g/l of D-glucose, 4 m/l of citric acid, 2 g/l of triaminomcitrate, 5 g/l of sodium acetate, 2 g/l of KH2PO4, 0.1 g/l of MgSO4, 0.08 g/l of MnSO4·5H2O, 0.02 g/l of bromocresol purple, and 15 g/l of agar (Nihon Pharmaceutical). Fecal samples were homogenized in 450 μl of 50 mM anaerobic phosphate buffer. A dilution series (10^-1~10^-6) was then prepared, and 30 μl of each dilution was plated onto LPSM. The plates were anaerobiocally cultivated at 37 °C for 2 d using the AnaeroPack system. After cultivation, the single colonies colored by a yellow-colored halo were counted. The number of L. plantarum per g of feces was calculated and is presented in logarithmic CFU.

In a separate experiment, mice acclimatized to the control diet were deprived of food overnight and administered 0.2 ml of PBS containing LP14 and rifampicin-resistant L. johnsonii (1 × 10^8 CFUs each) intragastrically. The mice were further deprived of food for 8 h after administration and were then re-fed the control diet. Feces were collected from individual mice at 0, 6, 24, 48, 72, and 120 h after administration. The recovery of viable L. plantarum and rifampicin-resistant lactobacilli was measured using LPSM and rifampicin-supplemented (100 μg/ml) MR medium respectively. After cultivation at 37 °C for 2 d under anaerobic conditions, single colonies were counted. The total number of L. plantarum and rifampicin-resistant lactobacilli per g of feces was calculated and is presented in logarithmic CFU.

Profile analysis of fecal microbiota by PCR-denaturing gradient gel electrophoresis. After feeding of each of the test diets for 28 d, DNA was isolated from fresh feces using the IsolPlant DNA isolation kit (Nippogence, Tokyo) following the manufacturer’s instructions. DNA samples served as a template to amplify the 16S rRNA gene fragments using universal primers U968-GC (GCC CGG GGG GGG CGG CCC GCC GGC GG GGG GGG GCA CGG GAA CGG GAA GCA CTT TAC) and L1401 (CGG TGT GTA CAC GAC CC), as reported previously.20 PCR was performed in a 25-μl reaction mixture containing 500 μM each of U968-GC and L1401, 1 × Green Go Taq Flexi buffer (Promega, Madison, WI), 1 mM MgCl2, 0.2 mM dNTP, 1.25 U of Go Taq Hot Start polymerase (Promega), and 5 μg/ml of DNA template. The reaction conditions were 94 °C for 5 min, followed by 35 cycles of 94 °C for 30 s, 56 °C for 20 s, and 68 °C for 40 s, and a final extension at 68 °C for 7 min. The amplicons were analyzed by denaturing gradient gel electrophoresis (DGGE) as described previously.20,21 The exception with the method that we used an 8% polyacrylamide gel that contained a 40% to 65% gradient of urea-formamide and SYBR gold nucleic acid gel stain (Molecular Probes, Eugene, OR) for gel staining. Quantity One software (version 4.6.0, Bio-Rad, Hercules, CA) was used for band identification and normalization of band patterns from the DGGE gels. A dendrogram of the DGGE-band profile was constructed using Pearson’s curve-based correlation and the unweighted pair-group method with the arithmetic mean (UPGMA) clustering method of Quantity One software, as previously described.21

In vitro culture experiments. Carbon source-free MRS broth was used as a basal medium to culture LP14. Then 1 × 10^5 CFU of bacteria were inoculated into 10 ml of basal medium, and the medium was supplemented with glucose, FOS, or inulin at a concentration of 15 g/l. Cultures were statically incubated at 37 °C under non-pH controlled conditions. The growth of LP14 was evaluated by measuring the optimal density (OD) at 600 nm with the pH of the cultures at 0, 3, 4, 5, 6, 7, 8, 9, 10, 11, and 12 h. In a separate experiment, LP14 was cultivated in fecal extract medium (FEM) prepared following the method of Pompei et al., with some minor modifications.14 Briefly, fecal pellets were collected from ddY mice fed each of the test diets for 28 d and were homogenized in distilled water. The homogenates were then centrifuged at 10,000 × g for 15 min and the precipitate was washed twice with distilled water. The supernatants at each washing step were collected, and the volume
was adjusted to a final dilution factor of 1:10 w/v. The sample was
then filtered through filter paper and a 1.2-μm size membrane, and
t-cysteine was added at a final concentration of 0.5 g/l and the pH
was adjusted to 7.0. After autoclaving at 110°C for 30 min, the samples
were subjected to culture experiments in FEM. In the mono-culture
experiments, LP14 (1.5 x 10⁹ CFU) was cultured in 5 ml of FEM. In
the co-culture experiments, LP14 (1 x 10⁹ CFU) was cultured with
cecal bacteria (1 x 10⁹ cells) prepared from mice fed the various test
diets in 5 ml of FEM. For preparation of fecal bacteria, fecal samples
were suspended in 1 ml of anaerobic PBS containing 1 mM dithio-
threitol and 0.01% w/v Tween 20, and homogenized by vortexing
for 3 min. After centrifugation at 700 x g for 1 min, the supernatant
was recovered and centrifuged at 6,000 x g for 3 min, and the resulting
pellet was used as fecal bacteria. Cultures were statically incubated
for 12 h at 37°C under non-pH controlled anaerobic conditions generated
by the AnaeroPack system. At the end of the culture period, the total
number and viability of the bacteria was measured by flow cytometry
following the method of Ben-Amor et al. Briefly, 10 μl of culture
was added to 1 ml of anaerobic PBS containing 1 mM dithiothreitol,
0.01% w/v Tween 20, 10⁶ particles/ml of fluorospheres (Flow-Check
fluorospheres, Beckman Coulter, Tokyo), 1 μg/ml of propidium iodide
(Wako) and 5 nM SYTO-BC (Molecular Probes). After 10 min of
incubation at room temperature, samples were analyzed by flow
cytometry (Epics XL, Beckman Coulter). The number of L. plantarum
after the co-cultivation period was measured by LPSM, as described
above.

Histological observation of fluorescein-stained bacteria in the gut.
LP14 was stained with PKH26 using the PKH26 Red Fluorescent Cell
Linker Kit (Sigma, St. Louis, MO) following the manufacturer’s
instructions. L. johnsonii was stained with 5-(6)-carboxyfluorescein
diacetate succinimidyl ester (CFSE, Enzo Life Sciences, Farmingdale,
NY). Briefly, 1 x 10⁹ CFUs of L. johnsonii was suspended in 1 ml
of PBS containing 25 μM CFSE. After incubation at room temperature for
10 min, a bacterial pellet was obtained by centrifugation at 10,000 x g
for 5 min and washed twice with PBS. DiY mice were fed the test diets
for 63 d and deprived of food overnight. Thereafter, the mice were
administered 0.2 ml of PBS containing both PKH26-stained LP14 and
CFSE-stained L. johnsonii (1 x 10⁹ CFU each) intragastrically. The mice
were deprived of food until the end of the experiment. At 8 h after
administration, they were anesthetized with diethyl ether and sacrificed
by exsanguination of the carotid artery. Following laparotomy, the
stomach, ileum, cecum, and colon were excised. Each tissue was then
washed with ice-cold PBS to flush out the luminal contents, fixed
in 4% w/v paraformaldehyde in 0.1M phosphate buffer (pH 7.4)
overnight, and embedded in OCT compound (Sakura Finetechnical,
Tokyo). Five-μm cryostat sections were prepared and stained with
4,6-diamidino-2-phenyl indole (DAPI; Molecular Probes) and observed under a fluorescence
microscope. The luminal contents obtained from each tissue were
also centrifuged at a final concentration of 10,000 x g for 5 min. The cell
pellet was stained with DAPI and observed under a fluorescence
microscope.

Statistical analysis. Results are presented as mean ± SEM. Tukey-
Kramer's test following one-way analysis of variance was used to
compare mean values among the three groups. Differences between
two groups were evaluated by unpaired t-test. Data analysis was
performed using StatView for Macintosh (version 5.0, SAS Institute,
Cary, NC). p values < 0.05 were considered statistically significant.

Results

Effects of inulin-type fructans on the in vitro growth of LP14
LP14 was cultured in medium supplemented with
and without glucose, FOS, and inulin as the sole
carbon source, and its growth was monitored by changes in OD at 600 nm and pH (Fig. 1A and B
respectively). In the medium that did not receive any
carbohydrate supplementation, the OD remained at a
low level throughout the culture period. In contrast, the
measured OD for the medium supplemented with
glucose began to increase at 4 h and reached a plateau
at 10 h. The OD levels continued to increase until the
end of the culture period for the medium supplemented
with FOS and inulin, but remained lower than in the
medium supplemented with glucose. The OD levels
appeared to be similar for the FOS- and the inulin-
supplemented medium throughout the culture period.
Although the pH levels showed minimal changes in the
medium that did not receive carbohydrate supplemen-
tation, the pH of the medium supplemented with
glucose decreased to a minimum level of approximate-
ly 4.0 at 11 h. The pH in the medium supplemented
with FOS and inulin also decreased, but the levels were
higher than those observed for the medium supple-
mnted with glucose.

Effects of fructan feeding on survival and persistence of LP14 in mice
The time course changes in fecal recovery of viable
L. plantarum following LP14 administration in mice fed
FOS and inulin for 2 d are presented in Fig. 2A. Prior to
LP14 administration, L. plantarum was not detected in
any of the mice. Fecal recovery of L. plantarum
increased after LP14 administration, peaking at 6 h and
decreasing to the levels observed before administration
by 48 h. At 24 and 30 h after LP14 administration, fecal
recovery was significantly higher in the mice fed the
inulin diet than in those fed the control diet. In addition,
fecal recovery in the mice fed the FOS diet was
significantly higher than in those fed the control diet at
30 h after administration. The calculated area under the curve (AUC) of the inulin diet-fed group was significantly higher than that of the other two groups (Fig. 2B).

Fecal recovery of L. plantarum after LP14 administration was also investigated in the mice fed the test diets for 28 d. In order to determine the effects of chronic FOS and inulin feeding on the composition of gut microbiota, analysis of the 16S rRNA gene present in the fecal DNA was done using PCR-DGGE. Figure 3 shows the DGGE band profile of all the mice included in the experiment. The intensity and position of the detected bands was then subjected to cluster analysis, where the band profiles were clearly divided into three clusters according to the test diets. Figure 2C reveals the time course changes in fecal recovery of L. plantarum following LP14 administration in mice fed FOS and inulin for 28 d. Similarly to mice fed the test diets for 2 d, L. plantarum was not detected in any of the mice before LP14 administration. Fecal recovery of L. plantarum increased after LP14 administration, peaking at 6 h and decreasing to the levels observed before administration by 48 h. At 6 h after LP14 administration, fecal recovery was significantly lower in the mice fed the inulin diet than in those fed the control diet. In addition, fecal recovery was significantly higher in the mice fed the FOS and inulin diets than in those fed the control diet at 30 h following LP14 administration. The AUC in the mice fed the inulin diet was significantly higher than in the mice fed the control diet (p = 0.127, Fig. 2D).

Fig. 2. Time Course Changes in Fecal Recovery of Viable Lactobacillus plantarum from ddY Mice after Lactobacillus plantarum No. 14 (LP14) Administration.

The mice were fed a control diet (◇) or a diet supplemented with fructo-oligosaccharide (FOS) (◆) or inulin (□) for 2 (A) or 28 d (C). The total number of viable L. plantarum in the feces was calculated using L. plantarum-selective medium (LPSM). The area under the curve in each group is presented in part B (2 d) and part D (28 d). Values are expressed as mean ± standard error. * Mean value is significantly different from control group (p < 0.05). † Mean value is significantly different from the FOS group (p < 0.05).

Fig. 3. Changes in Gut Microbial Composition in ddY Mice Fed a Control Diet or a Diet Supplemented with Fructo-Oligosaccharide (FOS) or Inulin for 28 d.

PCR-DGGE analysis of fecal DNA based on bacterial 16S rRNA gene sequences was performed, and the SYBR gold-stained DGGE fingerprint is shown. Similarities among DGGE band profiles were calculated based on the position and intensity of the bands, and the dendrogram of the DGGE band profiles was constructed by the UPGMA clustering method. Distances are measured in arbitrary units.
Evaluation of the adhesion of LP14 to the mouse gastrointestinal tract

Figure 4 shows the fecal recovery of L. plantarum and rifampicin-resistant lactobacilli after administration of LP14 and rifampicin-resistant L. johnsonii in the mice fed the control diet. L. johnsonii was used as a positive control bacterium that persistently colonized the mouse gut. Neither L. plantarum nor rifampicin-resistant lactobacilli were detected prior to administration. Following administration, fecal recovery of both L. plantarum and rifampicin-resistant lactobacilli increased to approximately $10^8$ CFU/g of feces at 6 h. After this time, the fecal recovery of L. plantarum decreased, and it was no longer detected after 48 h. Fecal recovery of rifampicin-resistant lactobacilli also decreased, but held at approximately $10^6$ CFU/g of feces over 120 h. Fecal recovery of rifampicin-resistant lactobacilli was maintained at significantly higher levels than those of L. plantarum over the experimental period.

Effects of fructan feeding on the in vitro growth of LP14 in fecal extract medium prepared from mice

LP14 was mono-cultured for 12 h in FEM prepared from the feces of mice fed the various test diets (Fig. 6A). The numbers of bacteria increased from $1.5 \times 10^5$ CFU to more than $1 \times 10^6$ cells in all FEM. More than 97% of these bacteria were viable. In comparison to FEM prepared from the mice fed the control diet, the total number of bacteria observed was significantly higher in the FEM prepared from the mice fed the inulin diet. The number of bacteria in the FEM prepared from the mice fed the FOS diet also tended to be higher than the FEM prepared from the mice fed the control diet ($p = 0.060$). In addition, LP14 ($1 \times 10^7$ CFU) was co-cultured for 12 h with fecal bacteria ($1 \times 10^2$ cells) from mice fed the various test diets in FEM (Fig. 6B). Both the total number of bacteria and L. plantarum in the FEM prepared from the mice fed the
inulin diet was significantly higher than the FEM prepared from the mice fed the control diet. In the FEM prepared from the mice fed the FOS diet, the total number of bacteria was significantly higher than the FEM prepared from the mice fed the control diet, whereas the number of L. plantarum tended to be higher than in the FEM prepared from the mice fed the control diet (p = 0.077). The viable population of total bacteria was approximately 90% in all FEMs.

Discussion

In the current study, we investigated to determine whether dietary supplementation with fructans would influence the growth and persistence of orally administered Lactobacillus strain LP14 in the murine gastrointestinal tract. We showed that consumption of fructans increased fecal excretion of viable LP14, suggesting that fructans stimulate the growth and survival of LP14 in the gut. This is consistent with numerous previously reported studies. Su et al. showed that supplementation with soybean oligosaccharide and inulin-type fructans increased fecal recovery of viable Lactobacillus acidophilus LAFTI L10, Bifidobacterium lactis LAFTI B94, and Lactobacillus casei L26 LAFTI in mice. Frece et al. also found that fecal counts of Lactobacillus helveticus M92 increased following dietary inulin, lactulose, and raffinose intake.

In our study, we also showed that the viable LP14 identified in fecal samples were reduced to an undetectable level at 48 h following administration of a fructan or a control diet. In contrast, rifampicin-resistant lactobacilli remained detectable (approximately 1 × 10^5 CFU/g feces) for at least 120 h after administration of rifampicin-resistant L. johnsonii isolated from the mouse stomach. These data suggest that orally administered L. johnsonii continued to be present in the feces. Thus it appears likely that the administered bacteria were able to colonize the gastrointestinal tract when these bacterial species were indigenous to the tract. However, non-indigenous species appeared less likely to colonize the gastrointestinal tract, even though prebiotics were administered simultaneously. Indeed, histological observation revealed that PKH26-stained LP14 were predominantly present in the luminal contents, even though a number of CFSE-stained L. johnsonii were associated with the non-secreting epithelium of the stomach.

In vitro culture experiments showed that inulin and FOS supported the growth of LP14 in similar manners. However, when LP14 was cultured in FEM prepared from mice, the growth of LP14 was highest in the FEM from the mice fed the inulin diet, followed by the FOS and control diets. These findings are consistent with fecal excretion of viable LP14 in the mice. In addition, the growth of LP14 in co-cultures with fecal bacteria was also higher in the FEM from the mice fed the fructan diet. These results suggest that dietary fructans stimulate the growth of LP14 in the gastrointestinal tract. Since dietary fructans are largely degraded by gut microbiota during passage through the gastrointestinal tract and are therefore virtually non-existent in fecal samples, the possibility that fructans excreted in feces support the growth of LP14 should be ruled out. It appears likely that some of the factors derived from fructan fermentation by gut microbiota might stimulate LP14 growth. Given that fermentation of inulin-type fructans by gut microbiota depends on their DP, the higher growth-stimulating potency of inulin as compared to FOS might be associated with the level of fermentation products. Because short-chain fatty acids (SCFAs) are predominant end products of fructan fermentation in the distal gut, our preliminary experiments examined the growth of LP14 in the medium supplemented with SCFA, but physiological concentrations of SCFA had no influence on the growth of LP14 in vitro (unpublished results). Further studies are thus needed to explore the factors that stimulate the growth of LP14 in the gastrointestinal tract.

16S rRNA-based PCR-DGGE analysis indicated that the composition of fecal bacteria differed among the mice fed the various test diets for 28 d. Even under these conditions, the fecal recovery of viable L. plantarum after administration of LP14 was higher in the mice fed the FOS and inulin diets as compared to the mice fed the control diet. These results suggest that dietary fructans stimulated the growth of LP14 even under conditions where dietary fructans modulate gut microbiota composition.

Fecal recovery of L. plantarum at 6 h after administration of LP14 was lower in the mice fed the inulin diet than those fed the control diet for 28 d. This was most likely the result of reduced gastric emptying and intestinal transit caused by chronic feeding of an inulin diet. Previous studies have found increases in peptide YY (PYY) and glucagon-like peptide-1 (GLP-1) in the
portal venous blood following fructan feeding in rats.28) Both PYY and GLP-1 are known to suppress gut motility.29) Delzenne et al. proposed that SCFA promotes L cell differentiation and proglucagon expression in L cells, leading to an increase in PYY and GLP-1 production.28) In the current study, nevertheless, the calculated AUC of fecal recovery of \textit{L. plantarum} was significantly higher in the mice fed the inulin diet than the mice fed the control diet. Thus it appears likely that dietary inulin stimulates the growth and survival of LP14 in the gut, even if fecal excretion is retarded by chronic consumption of inulin.

In combination, the present findings suggest that LP14 exhibits the ability to survive but not to colonize the mouse gastrointestinal tract, and that dietary inulin-type fructans maintain higher levels of LP14 by promoting growth in the gastrointestinal tract.

References