

Evaluation of Prebiotic Effects of High-Purity Galactooligosaccharides *in vitro* and *in vivo*

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Summary

Galactooligosaccharides (GOS) are an important class of dietary prebiotics that exert beneficial effects on intestinal microbiota and gut barrier function. In this study, high-purity GOS (HP-GOS) were investigated *in vitro* and *in vivo* and confirmed as prebiotic ingredients in rat diet. HP-GOS were successfully produced using a two-step process, enzymatic hydrolysis and fermentation by yeast. They were found to serve as a good substrate and carbon source for supporting the growth of probiotic bacteria more effectively than other commercial GOS. Following administration of 1 % (by mass) of HP-GOS to rats, the growth of *Bifidobacterium bifidum* and *B. longum* in the gut increased most rapidly up to 12 h, and thereafter the increase was slow. Therefore, 1 % HP-GOS was found to be acceptable for the growth of probiotic bacteria. Groups of animals that were orally administered HP-GOS and bifidobacteria during the study, and the group administered HP-GOS during the 2nd (days 13–15) and 4th (days 28–30) period of the study had significantly ($p < 0.05$) higher numbers of bifidobacteria in faeces than groups receiving a single dose of bifidobacteria. HP-GOS affected the expression of genes encoding glucagon-like peptide-1 (GLP-1) and peptide YY (PYY). There was a significant upregulation of GLP-1 and PYY mRNA with HP-GOS and bifidobacteria intake. We propose that the prebiotic properties of HP-GOS are potentially valuable for the production of functional foods for human consumption.

Key words: high-purity galactooligosaccharides, bifidobacteria, genes encoding GLP-1 and PYY peptides

Introduction

There has been an increasing interest in the regulation of colonic microflora in order to improve the host's health. This has been achieved traditionally by dietary inclusion of live microbes as food supplements known as probiotics. An alternative approach involves the consumption of food ingredients known as prebiotics. Prebiotics may provide advantages to probiotic bacteria in the gastrointestinal tract and additionally exert direct effects on the microflora in the large intestine (1).

Galactooligosaccharides (GOS) that consist of 3–10 molecules of galactose and glucose are known to facilitate the growth of desirable intestinal microflora and are considered as potent non-digestible prebiotics (2). Commercial GOS that contain complex mixtures of oligosaccharides with different glycosidic linkages and degrees of polymerization are usually synthesized by enzymatic transgalactosylation of lactose by β -galactosidases from various sources such as yeast, fungi or bacteria (3,4). In addition, these kinds of products can also contain transgalactosylated oligosaccharides, unreacted lactose, glu-

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cose and galactose, which do not have prebiotic properties but only the caloric value (5).

The most efficient process to produce high-purity GOS (HP-GOS) is yeast fermentation of sugars such as glucose, galactose and lactose (6). Cardelle-Cobas *et al.* (7) first reported the production of non-monosaccharides and HP-GOS by repeated batch fermentation with immobilized yeast cells. A GOS syrup of an increased purity was produced by immobilized β -galactosidase from *Penicillium expansum* F3 and subjected to fermentation by *Saccharomyces cerevisiae* L1 and *Kluyveromyces lactis* L3 (8). This was a feasible industrial process to produce high-purity GOS.

Among numerous non-digestible carbohydrate-based prebiotics, convincing scientific evidence for suitability for use as prebiotics exists only for inulin/oligofructose and GOS (9). Studies of the prebiotic effects of GOS and FOS in humans have shown that a daily dose of 4–20 g significantly increases the population of lactobacilli and bifidobacteria in the gut (10). Other effects, such as hypocholesterolemic effects, prevention of colon cancer, and enhancement of calcium absorption have been described (11,12). Numerous studies have reported data on the effects of non-digestible oligosaccharides (NDOs) and dietary fibre content on serum cholesterol and lipid levels; however, only a limited number of reports indicate positive effects of GOS (13) or inulin (14) on serum cholesterol metabolism in humans. Yamashita *et al.* (15) suggested first that dietary inclusion of fructooligosaccharides demonstrated hypocholesterolemic effect in diabetic subjects.

In this paper, the ability of HP-GOS to support the *in vitro* growth of selected strains of probiotic bacteria, namely *Lactobacillus acidophilus*, *Lactobacillus casei*, *Bifidobacterium bifidum* and *Bifidobacterium longum*, is investigated. In addition, it was determined whether oral administration of HP-GOS affected the growth of bifidobacteria, as well as serum cholesterol and nitrogen levels and the expression of gene encoding glucagon-like peptide-1 (GLP-1) and tyrosine-tyrosine peptide (PYY), which act as significant modulators of appetite *via* their peripheral effects (on the vagus nerve) and/or by influencing directly the arcuate nucleus in the rat.

Materials and Methods

Materials

HP-GOS, containing 75.18 % of galactooligosaccharides (by mass), were obtained from Neo Cremer Co. Ltd, Seoul, Republic of Korea. Commercial GOS, Y-GOS (52.52 % GOS, by mass; Yakult Honsha, Tokyo, Japan), C-GOS (56.25 % GOS, by mass; Doosan Corn Products Korea, Seoul, Republic of Korea), and Q-GOS (41.77 % GOS, by mass; Samyang Genex, Incheon, Republic of Korea) were purchased from a local market in Seoul, Republic of Korea. A commercial β -galactosidase from *Bacillus circulans* was obtained from Bision Corporation (Seongnam, Republic of Korea).

Production of GOS

Batch reactions were performed by incubating β -galactosidase with a 40–45 °Brix lactose solution in a 100-litre incubator shaker at 150 rpm. Lactose (25 kg) was dis-

solved in distilled water (60 L), and 0.08 % (by mass) β -galactosidase from *Bacillus circulans* was added to synthesize GOS at 55 °C and pH=6.0 for 24 h. All reactions were terminated by incubation at 100 °C for 10 min and the sugar profile was analyzed by high-performance liquid chromatography. To increase the GOS content, 100 mL of 20 % (by mass) GOS syrup produced by enzymatic hydrolysis with β -galactosidase were fermented by 8 % (by mass) of fresh yeast (*Saccharomyces cerevisiae* L1). The fermentation process was carried out in a shaking incubator at 100 rpm and 30 °C for 24 h. After each fermentation cycle, cells were transferred to 20 % (by mass) GOS syrup, which was then filtered and treated with active carbon for decolourization. Ion exchange chromatography using Amberlite® CG-120-II column (Sigma-Aldrich, Buchs, Switzerland) was utilized for further purification. The pooled fractions were evaporated to 45 °Brix and dried with a spray dryer. Lactose and HP-GOS were determined by an HPLC system with Waters® 2414 refractive index detector (RID) (Waters Corporation, Milford, MA, USA) equipped with YMC-Pack Polyamine II column (4 mm×250 mm; YMC Co. Ltd, Kyoto, Japan), column heater (30 °C), and RID detector. Acetonitrile (64 %) was used as mobile phase.

Bacterial strains and growth conditions

Lactobacillus acidophilus DDS-1, *L. casei* KCTC 12452, *Bifidobacterium bifidum* KCTC 3357 and *B. longum* SJ 32 were obtained from the culture collection from the Korea Research Institute of Bioscience and Biotechnology, Daejeon, Republic of Korea. The cultures were grown at 37 °C in modified peptone yeast extract fructose (PYF) medium without carbon source consisting of (in %, by mass): yeast extract 1, peptone 0.5, L-cysteine HCl 0.5 and salt solution 4 (containing (in %, by mass): CaCl₂ 0.02, MgSO₄ 0.02, K₂HPO₄ 0.1, KH₂PO₄ 0.1, NaCl 0.2 and Na₂HPO₃ 1.0) (16). The medium was supplemented with 0.5, 1, 2 or 4 % (by mass) GOS. Microplates were incubated anaerobically at 37 °C in a GasPak™ container (BD (Becton, Dickinson and Company), Franklin Lakes, NJ, USA). The absorbance at 600 nm ($A_{600\text{ nm}}$) was recorded by the microplate reader at 0, 24 and 48 h. At 24 h, after the measurement of $A_{600\text{ nm}}$ the plates were placed in the incubator to restore anaerobic conditions.

Animals and diet

The experimental protocol was reviewed and approved by Institutional Animal Care and Use Committee of Korea University. Four-week-old male Sprague Dawley® (SD) rats were purchased from Daehan Biolink Co. Ltd. (Cheongju, Republic of Korea). The animals were kept in a room at 24 °C and constant atmosphere with 60 % humidity and a 12-hour light/dark cycle. Rats were fed an AIN-93G diet based on the main ingredients of corn starch (40 %) and casein (20 %) (17). After an adaptation period, the rats were randomly divided into four groups (N=8): the control group (oral administration of saline), the HP-GOS group (oral administration of HP-GOS), the HP-GOS+BB group (oral administration of HP-GOS and bifidobacteria), and the BB group (oral administration of bifidobacteria). The groups were orally administrated HP-GOS (1.5 mL of the solution of 1 g of HP-GOS and/or 10⁹ CFU bifidobacteria) daily for 5 weeks.

Fresh faecal samples were collected weekly (equal mass from four rats per pool) in sterile flasks, and kept at -80°C for microbiological analysis. To count the total number of bifidobacteria, 3 g of faecal sample were diluted in 25 mL of dilution solution and an aliquot of 0.2 mL was spread on Petri dishes using BL agar. Colonies were incubated anaerobically during 2–3 days at 37°C under anaerobic conditions and results were measured as the log CFU per gram of faecal sample.

At the end of the study, the rats were euthanized using CO_2 asphyxiation and the liver, kidney and spleen were removed and weighed immediately. The body mass of each rat was measured every week for 5 weeks and the mass of each organ was expressed as 100 g of body mass.

Blood analysis

Blood samples were collected into non-heparinized serum separator tubes. Serum triglycerides (TGs), total cholesterol (TC), and high-density lipoprotein cholesterol (HDL-C) levels were measured by using a FUJI Dri-Chem 3500 system (Fuji Photo Film Co., Osaka, Japan). Concentration of low-density lipoprotein cholesterol (LDL-C), in mg per 100 mL, was calculated according to the method of Friedewald *et al.* (18) as follows:

$$\gamma(\text{LDL-C}) = \gamma(\text{TC}) - \gamma(\text{HDL-C}) - (\gamma(\text{TG})/5) \quad /1/$$

RNA extraction and real-time PCR

Total RNA was obtained from the intestine samples by using TRIzol reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's protocol. Reverse transcription was performed by using 1 μg of total RNA with first strand cDNA synthesis kit for real-time polymerase chain reaction (RT-PCR, Invitrogen) with oligo (dT) 15 as a primer (Invitrogen). After cDNA synthesis, real-time PCR was performed using a Power SYBR[®] Green PCR Master Mix kit (Applied Biosystems, Foster City, CA, USA). Quantitative analysis was carried out using StepOne plus software v. 2.0 (Applied Biosystems). Results were normalized to a validated control gene, β -actin, using the $\Delta\Delta\text{Ct}$ method (19). Using primers to interrogate proglucagon and PYY, RT-PCR was performed by a method reported previously (20). The following primers were used for GLP-1 and PYY: proglucagon (NM-012707.2): forward primer: 5'-ATGCGGACGAATACATTTCC-3', reverse primer: 5'-CTCAGGGCGGTAACCTCAAAA-3'; PYY (NM_001034080.1): forward primer: 5'-CAGCGGTATGGAAAAGAGA-3', reverse primer: 5'-CATGCAAGTGAAAGTCGGTGT-3'; β -actin (NM-031144.3): forward primer: 5'-GCTACAGCTTACCACCACA-3', reverse primer: 5'-TGCCGATAGTGATGACCTGA-3'.

Statistical analysis

All statistical analyses were performed using the Statistical Package for Social Sciences, v. 12.0 (SPSS Inc., Chicago, IL, USA). The statistical significance of differences was determined using one-way ANOVA at a significance level of $p < 0.05$. All data were significant at 95 % level and reported as the mean value \pm standard deviation (S.D.).

Results and Discussion

GOS content before and after enzymatic hydrolysis and fermentation

Table 1 summarizes the content of saccharides during different stages of GOS preparation, *i.e.* the enzymatic hydrolysis by β -galactosidase and fermentation by *S. cerevisiae*. After treatment by β -galactosidase, GOS content increased from 0 to 51.0 g per 100 g, and lactose mass fractions decreased from 99.2 to 21.3 g per 100 g (Table 1). Monosaccharide (glucose and galactose) mass fractions also increased from 0.8 to 27.7 g per 100 g after the enzymatic reaction; however, these monosaccharides were completely removed by fermentation with *S. cerevisiae*, whereas lactose levels were slightly increased. In order to increase the purity of GOS, fermentation process was utilized. After fermentation, glucose and galactose mass fractions decreased from 27.7 to 0.0 g per 100 g (Fig. 1 and Table 1). The purity of the GOS preparation increased

Table 1. Saccharide content during galactooligosaccharide (GOS) production

Process	$w(\text{monosaccharide})$ g/100 g	$w(\text{lactose})$ g/100 g	$w(\text{GOS})$ g/100 g
Raw material	0.8 \pm 0.1	99.2 \pm 2.6	0.0
After enzyme reaction	27.7 \pm 1.8	21.3 \pm 1.0	51.0 \pm 3.3
After fermentation with <i>S. cerevisiae</i> L1	0.0	26.4 \pm 1.3	73.6 \pm 2.1

Values are expressed as mean \pm standard deviation

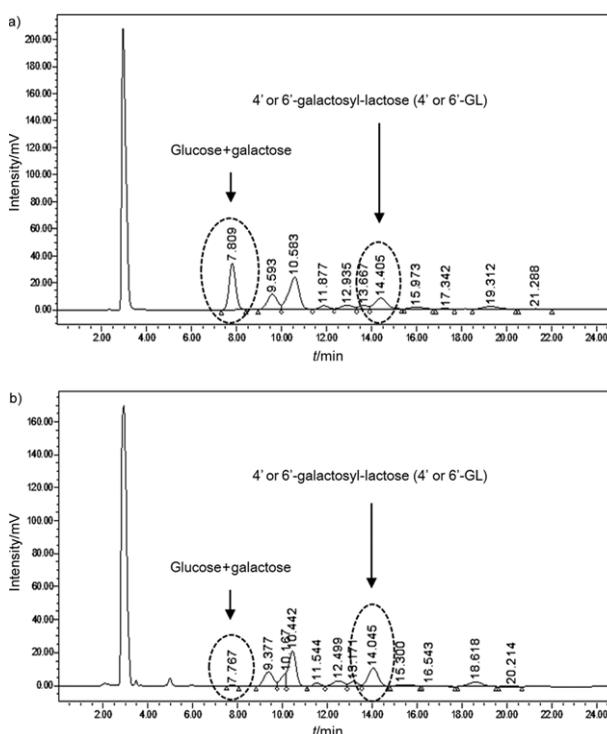


Fig. 1. Carbohydrate profile: a) before and b) after the fermentation with *Saccharomyces cerevisiae* L1

from 51.0 to 73.6 g per 100 g after fermentation with *S. cerevisiae* (Table 1), verifying that HP-GOS were successfully produced using the two-step enzymatic hydrolysis and fermentation by yeast.

For the production of HP-GOS, selective fermentation for a given microorganism was characterized. During the fermentation, ethanol may be produced depending on the used microorganism, which at toxic volume fractions may compromise the activity of the microorganism. *S. cerevisiae* was used to increase the purity of the mixture of GOS obtained by enzymatic hydrolysis with β -galactosidase from *Bacillus circulans*, with complete removal of the monosaccharides (21). The same approach was adopted for the production of GOS mixture from *B. bifidum* biomass (9). In a previous research, *Kluyveromyces marxianus* improved the purity of a GOS mixture produced by β -galactosidase from *B. circulans* from 38 to 97 % by selective fermentation of mono- and disaccharides (including lactose) (6). With a combined use of *S. cerevisiae* and *K. lactis* the purity of a GOS mixture produced by β -galactosidase from *Penicillium expansum* increased from 29 to 98 % (8). In this work, HP-GOS were produced by fermentation with *S. cerevisiae* (Table 1 and Fig. 1), which increased the GOS content from 51.0 to 73.6 g per 100 g after fermentation with *S. cerevisiae*.

Utilization of GOS by intestinal bacteria *in vitro*

HP-GOS comprise substances with structural differences and have considerably greater prebiotic potential compared to a commercially available GOS mixture. Fig. 2 shows the growth of intestinal bacteria in a medium

containing various commercial GOS. In order to confirm that the growth was dependent on GOS utilization, strains were also inoculated into PYF basal medium containing 1 % HP-GOS, Y-GOS, C-GOS or Q-GOS as the carbohydrate source. During the early stages of growth (after 12 h), all strains exhibited a similar growth rate without significant differences. All strains (*L. acidophilus*, *L. casei*, *B. longum* and *B. bifidum*) in a medium containing HP-GOS had a higher cell growth rate than the strains grown in the media containing commercial GOS after 12 h of culture. These results suggest that HP-GOS serve as a good substrate and carbon source for supporting the growth of probiotic bacteria.

Growth curves for the given strains grown anaerobically in the medium without GOS or with 0.5, 1, 2 or 4 % (by mass) GOS are shown in Fig. 3. *L. acidophilus* and *L. casei* were able to grow at all the tested GOS mass fractions, both reaching a maximum $A_{600\text{ nm}}$ of 1.15 after 36 h of growth. Cell growth was increased with an increase in HP-GOS mass fraction. Growth of *B. bifidum* and *B. longum* increased rapidly until 12 h and then continued increasing slowly. Therefore, HP-GOS mass fractions above 1 % were found to be acceptable for the growth of probiotic bacteria.

Structural differences of GOS vary notably depending on the conditions and source of enzymes used for their synthesis (HP-GOS and Y-GOS were produced from 4'- or 6'-galactosyl-lactose, and C-GOS and Q-GOS from 4'-galactosyl-lactose) (4), which affects the fermentation process as well as their prebiotic properties. Studies of

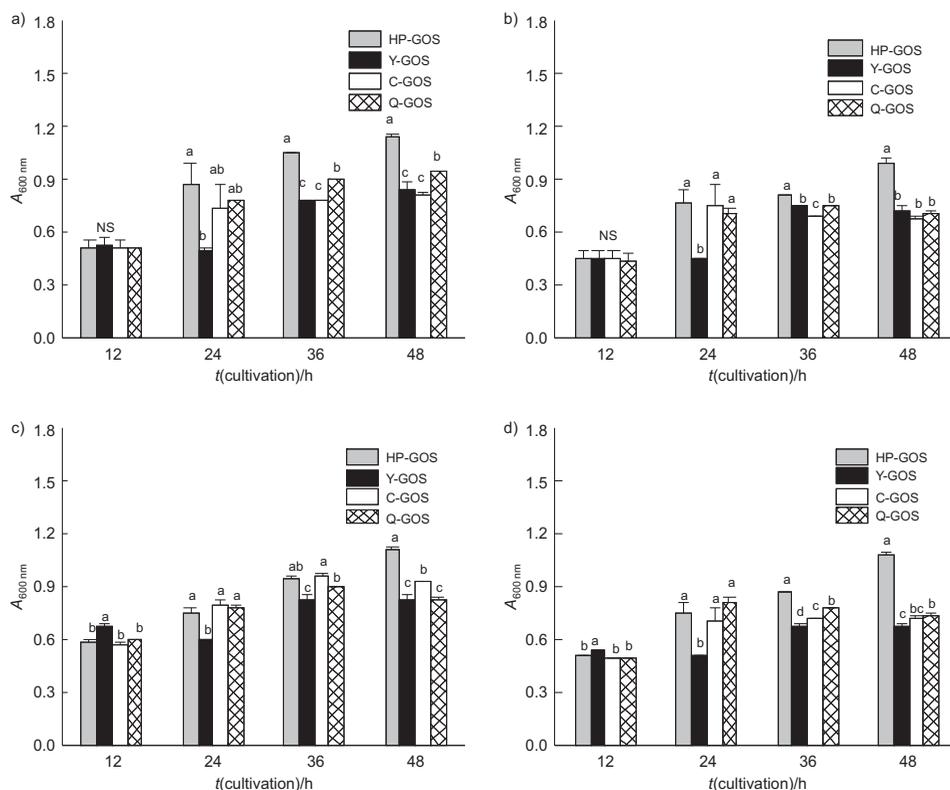


Fig. 2. Growth of: a) *L. acidophilus*, b) *L. casei*, c) *B. bifidum* and d) *B. longum* in modified PYF broth containing 1 % (by mass) of various commercial galactooligosaccharides (GOS). Bars represent the standard error of triplicate measurements. Different letters indicate significant differences at $p < 0.05$

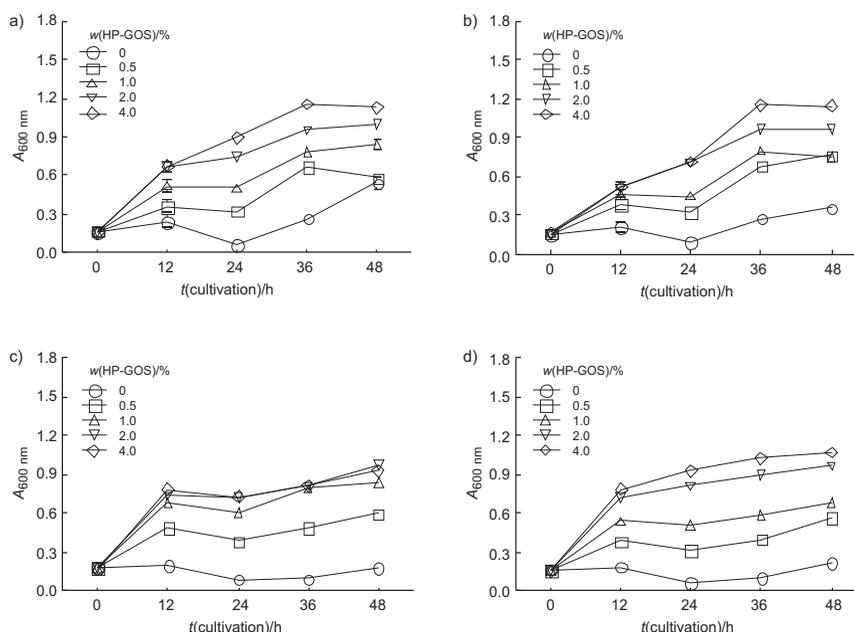


Fig. 3. Growth of: a) *L. acidophilus*, b) *L. casei*, c) *B. bifidum* and d) *B. longum* in modified PYF broth containing various mass fractions of high-purity galactooligosaccharides (HP-GOS). Bars represent the standard error of triplicate measurements

GOS utilization by bacteria have shown that different strains vary in their ability to ferment GOS, with individual strains exhibiting specific substrate preferences (22, 23).

We additionally analyzed the utilization of GOS by various probiotic bacteria (Fig. 2). *L. acidophilus*, *L. casei*, *B. bifidum* and *B. longum* had a higher cell growth rate when utilizing HP-GOS in comparison with commercial GOS (Fig. 2), suggesting that 4'- or 6'-galactosyl-lactose may be a more suitable substrate for these strains. The utilization of GOS by a number of probiotic bacteria has been extensively analyzed (24,25). It has been shown that 4'-galactosyl-lactose is selectively utilized by all the *Bifidobacterium* strains tested, compared with lactulose and raffinose, whose specificity is less noticeable. Other studies have also shown that some strains of *Lactobacillus*, *Bacteroides* and *Clostridium* ferment GOS, and that transgalactosylated disaccharides may even be better substrates for these bacteria (26). Some bacterial species can ferment both 4'- and 6'-galactosyl-lactose, although there are some differences and the growth of bacteria is dose-dependent.

As shown in Fig. 3, *B. bifidum* and *B. longum* utilized HP-GOS more rapidly than *L. acidophilus* and *L. casei*. It is considered that the utilization of non-digestible oligosaccharides (NDOs) by bifidobacteria is mediated by the hydrolyzing enzymes produced by these strains. Many *Bifidobacterium* strains produce glycolytic enzymes that hydrolyze a wide range of monosaccharides and various glycosidic bonds, while the activities of the enzymes from other enteric bacteria such as *Lactobacillus*, *Escherichia coli* and *Streptococcus* are less varied and are weaker than those from *Bifidobacterium* (27).

Changes in body mass, organ mass and serum parameters

Changes in body mass and organ mass of the rats in control, HP-GOS, HP-GOS+BB and BB groups are shown

in Table 2. No significant differences in body mass were found among the three groups. The changes in the masses of the liver and other internal organs of the rats after a 5-week administration of each diet are shown in Table 3.

Table 2. Changes in body mass of rat after treatment with galactooligosaccharides and/or bifidobacteria

Group	t/week				
	1	2	3	4	5
	m/g				
Control	44.7±1.0	90.8±2.7	147.7±5.2	201.4±7.4	230.5±6.1
HP-GOS	42.5±2.0	82.0±2.2	129.5±4.7	176.5±6.6	210.6±7.0
BB	43.5±1.5	88.3±3.3	139.6±3.1	193.0±7.9	219.0±8.2
HP-GOS+BB	45.2±1.9	90.3±3.3	147.0±5.1	196.6±5.8	224.3±6.0

Values are expressed as mean±standard deviation. Control=oral administration of saline, HP-GOS=oral administration of high-purity galactooligosaccharides, BB=oral administration of bifidobacteria, HP-GOS+BB=oral administration of HP-GOS and bifidobacteria

Table 3. Changes of organ mass of rat after treatment with galactooligosaccharides and/or bifidobacteria

Organ	w/(g/100 g)			
	Control	HP-GOS	BB	HP-GOS+BB
Liver	9.0±0.1	8.4±0.5	8.7±0.4	8.9±0.2
Kidney	2.3±0.1	2.1±0.1	2.3±0.1	2.3±0.2
Spleen	0.7±0.0	0.6±0.0	0.8±0.0	0.7±0.0

Values are expressed as mean±standard deviation. Control=oral administration of saline, HP-GOS=oral administration of high-purity galactooligosaccharides, BB=oral administration of bifidobacteria, HP-GOS+BB=oral administration of HP-GOS and bifidobacteria

No differences were observed in the masses of the liver, spleen and kidney in relation to the body mass in all groups.

Serum glucose, protein, aspartate aminotransferase (AST), alanine aminotransferase (ALT) and lipid profiles are shown in Table 4. Groups administered HP-GOS+BB had significantly different AST and ALT levels compared with the control group or the group administered HP-GOS, respectively ($p < 0.05$). However, the ALT and AST levels of the group administered HP-GOS+BB were in the normal range for SD rats (17.30–19.77 and 74.4–80.4 U/L, respectively) (28). There were no significant differences in the lipid profiles (total cholesterol, HDL cholesterol and triacylglycerol) among the groups.

Enumeration of bifidobacteria in rat faeces

Fig. 4 shows the total bifidobacteria counts in rat faeces, expressed in log CFU per g of faeces, during four periods of the study. The groups that were orally administered HP-GOS and bifidobacteria had significantly ($p < 0.05$) higher counts during all four periods, while the group receiving HP-GOS had higher counts in the 2nd and 4th period only in comparison with the control and BB group. In the 2nd and 3rd periods, the same trend was observed in groups administered HP-GOS and HP-GOS+BB. During the entire period, oral administration of HP-GOS+BB resulted in higher bifidobacteria counts than the oral administration of control or single administration of bifidobacteria. There was no significant difference in bifidobacteria counts between the HP-GOS+BB group and the HP-GOS group in the 2nd and 4th periods.

Bifidobacteria in rat faecal samples were counted in the HP-GOS groups with or without bifidobacteria. Our results indicated a positive effect in the synbiotic group (HP-GOS+BB) during the test periods. It is known that most bifidobacteria strains of human intestinal origin can readily use galactooligosaccharides; however, only a few strains from other genera, such as lactobacilli, possess this ability (24,26). As far as the effects of probiotic consumption on the bifidobacterial population are concerned, similar results have been observed in children and adults. Benno and Mitsuoka (29) reported an increase in the counts of bifidobacteria as well as a remarkable decrease in the counts of clostridia in adult human subjects consuming a daily dose of *B. longum*.

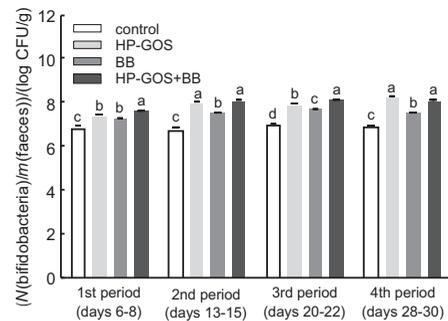


Fig. 4. Number of total bifidobacteria in rat faeces, expressed as mean values of log colony-forming units (CFU) per g of wet faeces, $N=6$. Bars represent the standard error of triplicate measurements. Different letters indicate significant differences at $p < 0.05$. HP-GOS=high-purity galactooligosaccharides, BB=bifidobacteria

Expression of genes encoding GLP-1 and PYY in ileum

There was an approx. 1.6-fold increase in PYY mRNA levels in the ileum of rats administered GOS+BB (Fig. 5), which was significantly higher than in the other groups ($p < 0.05$). Similarly, GLP-1 mRNA levels in the ileum of rats administered GOS+BB and only bifidobacteria were 1.5- and 1.6-fold higher, respectively, than in rats fed normal diet. There was a significant upregulation of GLP-1 and PYY mRNA with GOS+BB intake.

Endocrine cells in the intestinal mucosa secrete peptides involved in the regulation of food intake and/or pancreatic function; the latter are known as incretins (30,31). Endocrine L cells are distributed throughout the intestinal tract, and are predominantly present in the caeco-colon, where fermentation of inulin-type fructans occurs (32). GLP-1 and PYY, which are released from intestinal L cells, modulate appetite and thus reduce food intake (33). The ability of prebiotic fibre to increase the proglucagon mRNA levels and GLP-1 secretion is well supported (34,35). Increased proglucagon expression typically takes place when caecal mass is increased due to the markedly increased bacterial fermentation that occurs with the consumption of prebiotics (34,35). It is interesting to note that the fibre-containing prebiotic diet appears to have acute and lasting effects on the ability of L cells to produce and secrete GLP-1 and PYY. In the current study, measurements of GLP-1 and PYY levels were performed

Table 4. Serum levels of glucose, total protein, AST, ALT, and lipid profiles of rats

Group	γ (glucose) mg/dL	γ (total protein) g/dL	AST U/L	ALT U/L	γ (TC) mg/dL	γ (HDL-C) mg/dL	γ (LDL-C) mg/dL	γ (TG) mg/dL
Control	110.7±2.6	18.62±0.07	74.4±2.2 ^b	18.6±0.6 ^{ab}	79.2±2.5	52.7±2.8	11.4±3.0	75.3±3.0
HP-GOS	111.5±5.4	19.77±0.09	79.6±0.8 ^{ab}	19.8±0.7 ^a	83.8±2.5	56.2±2.3	14.6±1.6	77.7±1.7
BB	104.0±1.4	18.42±0.09	75.2±1.6 ^{ab}	18.4±0.9 ^{ab}	78.7±1.6	54.6±2.0	10.5±3.9	73.5±1.7
HP-GOS+BB	114.5±4.4	17.30±0.08	80.4±1.7 ^a	17.3±0.6 ^b	85.3±0.6	56.0±1.5	16.0±0.8	77.8±1.3

Values are expressed as mean±standard deviation, $N=8$. Mean values with different letters in superscript within the column are significantly different at $p < 0.05$ by Duncan's multiple range tests. AST=aspartate aminotransferase, ALT=alanine aminotransferase, TC=total cholesterol, HDL-C=high-density lipoprotein cholesterol, LDL-C=low-density lipoprotein cholesterol, TG=triglyceride. Control=oral administration of saline, HP-GOS=oral administration of high-purity galactooligosaccharides, BB=oral administration of bifidobacteria, HP-GOS+BB=oral administration of HP-GOS and bifidobacteria

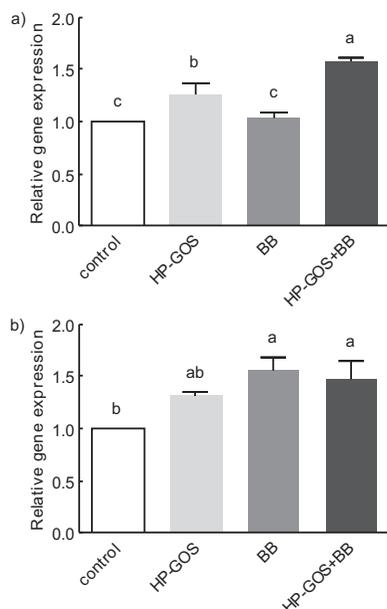


Fig. 5. Expression of genes encoding PYY and GLP-1 in the ileum of mice orally administered saline (control), high-purity galactooligosaccharides (HP-GOS), bifidobacteria (BB), and high-purity GOS+bifidobacteria (HP-GOS+BB) for 4 weeks, $N=8$. Bars represent mean values \pm standard error of the mean (SEM). Different letters indicate significant differences at $p < 0.05$

at the end of a 5-week period, when GOS as prebiotics were administered as part of the diet; however, we additionally found higher expression of genes encoding GLP-1 and PYY in the HP-GOS group with or without *B. bifidum* at the end of the period in which the high fat-based diet was administered (Fig. 5).

It is acknowledged that an optimum balance in microbial populations in the digestive tract is associated with good nutrition and health, and that this may be achieved by the consumption of probiotics. In particular, HP-GOS exhibit a greater prebiotic activity than other commercial GOS.

Conclusion

High-purity galactooligosaccharides (HP-GOS) were successfully produced using a two-step process, enzymatic hydrolysis and fermentation by yeast. They were found to serve as a good substrate and carbon source for supporting the growth of enteric bacteria, compared with other commercial GOS. The beneficial effect of regular intake of HP-GOS is attributed to the intestinal survival of probiotic *Lactobacillus* and *Bifidobacterium* strains. The health benefits associated with the consumption of HP-GOS in humans include improvement of intestinal tract health, increase in the expression of genes encoding GLP-1 and PYY, decrease in the risk of certain cancers, blood pressure control, and reduction of serum cholesterol levels. On the basis of our findings, we propose that the prebiotic properties of HP-GOS are valuable in the production of potential health-enhancing foods and supplements, and that HP-GOS may be used as a functional food ingredient for human consumption.

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Disclosures

None of the authors of this study has any financial interest or conflict with industries or parties.

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