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Evaluating prebiotic property of galactooligosaccharide produced by *Lactobacillus pentosus* var. *plantarum* BFP32 in fecal batch culture

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Abstract

Galactooligosaccharides (GOS) are one in prebiotic groups that composed of a chain of 2–9 galactose units and a terminal glucose. GOS was undigested carbohydrate that could pass through the small intestine in humans to reach the colon. GOS could be fermented by the useful microorganisms residing in human intestine. Bifidobacteria and lactobacilli are generally accepted beneficial contributions to the gut health of the human. This study aims to evaluate prebiotic property of the galactooligosaccharide produced by *L. pentosus* var. *plantarum* BFP32 in fecal batch culture. Production of GOS was achieved by enzymatic reaction of 60% lactose (w/v) as substrate and 100 U/ml of β-galactosidase at pH 7, 50°C. The mixture of GOS was further purified by yeast (*Saccharomyces cerevisiae* TISTR 5019) fermentation. The purified GOS syrup was freeze dried and chemical composition of the GOS powder revealed that it had 14% GOS with DP of 3 and 4. Evaluation of prebiotic property in fecal batch culture was performed and it was found that the GOS produced had prebiotic property with prebiotic index (PI) value of 1.19 which comparable to commercial GOS. There was also short chain fatty acids (SCFA) produced including lactic, acetic, butyric and propionic acid at highest concentrations of 5.30±1.57, 31.63±1.22, 42.18±1.49, and 2.37±0.56 µg/ml, respectively. Interestingly, butyric acid produced by fecal fermentation of the GOS in this study showed approximately twice higher than commercial GOS. Vitamins B1 and B2 were also produced at concentrations of 7.39±0.52 and 0.64±0.13 µg/ml, respectively.

Introduction

Prebiotics are non-digestible oligosaccharides that beneficially affect the host by stimulating the growth and/or activity of one or a limited number of bacteria in the colon, thus improving the host's health (Gibson and Roberfroid, 1995). A substantial amount of research on human clinical trials has indicated that prebiotics may indeed prove to be a clinically beneficial dietary supplement (Lewis and Atkinson, 2009). The benefits of prebiotics have been proven as anti-colon cancer, anti-pathogens in gastrointestinal tract, giving increased calcium absorption, and improved microbial balance in the gut (Vernazza *et al*., 2006; Wichienchot and Chinachoti, 2011). The non-digestible and fermentable oligosaccharides are the most popular compounds which gained specific interest because it had been reported to possess significant prebiotic properties such as galacto-oligosaccharides (GOS), fructo-oligosaccharides (FOS), gentio-oligosaccharides, gluco-oligosaccharides, isomalto-oligosaccharides (IMO), mannan-oligosaccharides, chito-oligosaccharides (COS), melibiose, pectic oligosaccharides (POS), and xylo-oligosaccharides (XOS) (Gibson *et al*., 2004; Roberfroid, 2007; Calame *et al*., 2008; Fastinger *et al*., 2008). Consumption of these prebiotics provide a beneficial physiological effect on the host by selectively stimulating the favorable growth or activity of a limited number of indigenous bacteria, namely bifidobacteria and lactobacilli (Gibson and Roberfroid, 1995). Metabolites from fermentation of prebiotics by microbiota are short chain fatty acids (SCFA), principally, acetate, propionate and butyrate and vitamins B and folic acid (Cummings, 1981).

Galactooligosaccharides (GOS) have received a lot of attention recently due to several health benefits associated with their consumption in particular dairy products. Galactooligosaccharides (GOS) are composed of a chain of 2–9 galactose units and a terminal glucose. GOS are considered prebiotic compounds (Gibson *et al*., 2004) since they pass undigested through the small intestine in humans to reach the colon, where they become selectively

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metabolized by some intestinal bacteria population, as bifidobacteria and lactobacilli, promoting their growth and/or activity. Intestinal lactobacilli and bifidobacteria have become of great interest due to their generally accepted beneficial contribution to the health of the host (Tuohy *et al.*, 2005). In addition, GOS may protect against intestinal pathogens via their anti-adhesive activity of enteric pathogens on the surface of gastrointestinal epithelial cells (Shoaf *et al.*, 2006). Throughout the past four decades, there has been a lot of development in the synthesis of these prebiotic compounds (Osman, 2015). Commercial GOS are usually synthesized by enzymatic transgalactosylation reaction from lactose by β-galactosidases from yeast, fungi or bacteria, obtaining complex mixtures of oligosaccharides with different glycosidic linkages and degree of polymerization (DP), depending on the source of the enzymes and experimental conditions (Ekhart and Timmermans, 1996; Otieno, 2010; Bruno-Barcena and Azcarate-Peril, 2015). In addition, these commercial products also contain glucose, galactose and lactose, without prebiotic properties, which increase the calorific value of the product. Until recently, there is a great interest in obtaining new prebiotic carbohydrates with improved properties, addressed to reach the distal regions of the colon unaltered to promote the growth of specific bacteria. In the human colon, it is diverse microbial ecology which affected by the diet. After diet had passed through mouth to the small intestine and large intestine. The food residues remain about 200 grams which around 60% of fecal dry weight are microbes.

The gut microbiota able to ferment carbohydrate and create organic metabolites and gases in the human colon. Fecal bacteria producing acetate are Bacteroides, Bifidobacteria, Eubacteria, Lactobacilli, Clostridia, Ruminococcus, Peptococci, Veillonella, Peptostreptococcus, Propionibacteria, Fusobacteria and Butyrivibrio. Propionate producers are Bacteroides, Propionibacteria and *Veillonella*. Butyrate can be produced by Clostridia, Fusobacteria, Butyrivibrio, Eubacteria and Peptostreptococcus. Fecal bacteria producing ethanol, succinate, lactate and pyruvate are Bacteroides, Bifidobacteria, Lactobacilli, Eubacteria Peptostreptococcus, Clostridia, Ruminococcus, Actinomycestes, Enterococci and Fusobacteria. Hydrogen can be produced by Clostridia, Ruminococcus and Fusobacteri (Egert *et al.*, 2006). By the way, production of SCFA by the microbial can promote benefits of human health, decrease the risk of colon cancer (particular butyrate) and stimulate immune response of human (Macfarlane *et al.*, 1998).

Materials and Methods

Microorganism and chemicals

Lactobacillus pentosus var *plantarum* BFP32 was used in this study (Hemmaratchirakul *et al*., 2013). The bacterial was cultured in MRS broth for 18 hours and centrifuged at 8,000×g to obtain cell paste. Cell suspension was prepared and cells were broken by acetone:toluene (9:1) solution. The β-galactosidase was extracted and it was used for synthesis of galactooligosaccharide. Galactooligosaccharide (GOS) was produced by 60% of lactose (Sigma-Aldrich Co. Ltd.) mixed with 100 U/ml β-galactosidase. The GOS was evaluated on prebiotic property in fecal batch culture. The sources of gut microbiota was obtained from fresh human feces. The donors of feces are who has healthy, don't received antibiotics, food or dietary supplement containing probiotic or prebiotic within last 3 months. Because it can affect changes of population numbers and diversity of gut microbiota.

Determination of sugar composition and degree of polymerization

Mono-, di- and oligosaccharide were analyzed by using HPLC systems. The sample was filtered through a 0.2 µm membrane and 5 µl samples were injected in a Rezex RNM column (7.8×300 mm, 5 µm). The mobile phase was HPLC-grade water and the flow rate was 0.4 ml/min. A refractive index (RI) detector was used and the column temperature was controlled at 80°C. Glucose and galactose were used as the monosaccharide standards and lactose was used as the disaccharide standard. The qualitative analysis of sugar in the sample was determined by comparison to the retention time of standard sugars. The concentration of sugar in the sample was calculated by comparing the peak areas of the standard curves of the respective sugars. Degree of polymerization (DP) of oligosaccharide was calculated regarding to their respective retention time. The molecular weight distribution of the mixed oligosaccharides was determined after the removal of the low molecular weight sugars (glucose, galactose and lactose) by yeast fermentation. The retention time of sample was compared to those of the standard curve (Mountzouris *et al.*, 1999). The oligosaccharides yield was calculated by the summary of the percentage area of the oligosaccharide fraction (Wichienchot *et al*., 2010).

Production of galactooligosaccharide

Lactose at 60% was used as a substrate for production of galactooligosaccharide with

optimum temperature of 50°C and pH 7.0. Source of β-galactosidase was *Lactobacillus pentosus* var. *plantarum* BFP32 isolated from fermented sausages. It was prepared according to the method of Hemmaratchirakul *et al*. (2013). The reaction was run for 8 hours and then stopped at 95°C for 15 minutes. The analysis of sugar content was performed by high performance liquid chromatography (HPLC) as mentioned above.

Purification of galactooligosaccharide

The GOS mixture was purified by *Saccharomyces cerevisiae* TISTR5019 for elimination of monosaccharide (glucose and galactose). *S. cerevisiae* could metabolize glucose for production of alcohol and could utilize galactose by the enzymes of Leloir pathway. Yeast (*Saccharomyces cerevisiae* TISTR5019) fermentation of GOS mixture was carried out in a rotary shaker at 180 rpm for 24 hours. The reaction was then stopped and elimination of alcohol was performed by heating the GOS mixture at 95oC for 30 minutes. Sample was taken for analysis of sugar content by high performance liquid chromatography (HPLC).

Fecal slurry preparation

The fecal slurry was prepared from fresh stool obtained from healthy donors who have no history used of antibiotics and consumption of food or dietary supplement containing probiotics and prebiotics within last 3 months. The fresh stool was weighted and diluted with 0.1 M phosphate buffered saline (PBS), pH 7 and mixed in stomacher for 2 minutes. Fecal slurry (10%) was prepared for use as inoculum in fecal batch culture.

Colonic fermentation by gut microbiota from fecal slurry

The basal medium was prepared, sterile and poured into a glass vessel of pH-controlled batch culture. The batch culture was performed under conditions stimulated human colon at 37°C, controlled pH at 6.8±0.2 with a pH controller. Each vessel was magnetically stirred and maintained under anaerobic conditions with oxygen-free nitrogen gas over time during fermentation for 24 hours. After inoculation (20 ml) of fecal slurry in a glass vessel containing basal medium (180 ml) and left the batch system stand for over-night without addition of GOS or sugar sample. Then, the GOS or sugar sample was added (4 grams) in the glass vessel. The pH of the mixture was controlled at 6.8±0.2 using an automated pH controller. The temperature of culture was controlled at 37°C by a circulating warm water though water-jacket of glass vessel. The samples were collected at 0, 3, 9, 12 and 24 hours for enumeration of bacteria, and analysis of short chain fatty acids and vitamins.

Microbial colony counting with fluorescent in situ *hybridization (FISH) technique*

The 375 microliter of sample was mixed with 1,125 µl of 4% (w/v) paraformaldehyde solution (pH 7.2). Sample was keep at 4°C overnight to fixed cell of the microbial. After that, the fixed cell was washed with PBS for 2 times. Sample was mixed with 150 µl PBS, 150 µl cold ethanol (96%, v/v), vortexed and stored at 20°C for at least 1 hour or until the need to use but should no longer than 3 months. For hybridization procedure, fixed cell sample was diluted to optimum concentration. Fixed cell sample (20 µl) was spread on the hole of slide coating with TEFLON/Poly-L-Lysine. The slide was placed on slide warmer at 45°C, for 10-12 minutes until the sample was dried. The slide was dipped into sequential ethanol solutions at concentration of 50, 80 and 96% (v/v). Pre-warmed hybridization buffer was prepared at the specific temperature for each probe. DNA probe for hybridize of bacteroides (Bac303) had sequence of CCAATGTGGGGGACCTT and its optimal hybridization temp was 48°C. Bifidobacteria (Bif164) had sequence of CATCCGGCATTACCACCC. Lactobacillus (Lab158) had sequence of GGTATTAGCA(T/C) CTGTTTCCA. Clostridia (Chis150) had sequence of TATGCGGTATTAATCT(C/T) CCTTT. Eubacteria (Eub 338) had sequence of GCTGCCTCCCGTAGGAGT (Rycroft *et al*., 2001). Sample (45 µl) was mixed with DNA probe (5 µl) that specific to each genus of bacteria. The DNA probes used for enumeration of each bacterial genus were Bif 164 for *Bifidobacterium* spp., Bac 303 for Bacteroidaceae and Prevotellaceae, Lab 158 for Lactobacillus-Enterococcus group, Chis 150 for *Clostridium histolyticum* group (Clostridium cluster I and II) and Eub 338 for Eubacteria or total bacteria (Rycroft *et al.*, 2001). Sample was placed into the slide and incubated in hybridization oven with optimum temperature of each probe for 4 hours. The slide was washed with washing buffer (50 ml) in the optimum temperature of each probe; Bac303 at 48°C, Bif164 at 50°C, Lab158 at 50°C, Chis150 at 50°C and Eub338 at 48°C for 15 minutes. After that the slide was washed with cold distilled water (50 ml), dried the slide and anti-fade solution (5 µl) was added. The slide was covered with cover slide and each genus of bacteria was counted under fluorescence microscope for 15 fields per hole. Prebiotic index (PI) was calculated by the following equation (Palframan *et*

al., 2003).

Prebiotic index (PI) = α+ β-γ–δ
α= (Bif_{24}/Bif_0)/Total
β= (Lac_{24}/Lac_0)/Total
γ= (Bac_{24}/Bac_0)/Total
δ= $(Clos_{24}/Clos_0)$/Total
Total = Eub_{24}/Eub_0

Eub_0, Eub_{24} was the quantity of Eubacteria or total bacteria at time 0 and 24 hours of fermentation, respectively.

Bif was Bifidobacteria; Lac was Lactobacilli; Bac was Bacteroides; Clos was Clostridium.

Whereas α and β had positive effect meanwhile γ and δ had negative effect.

Analysis of short chain fatty acid (SCFA)

The sample from batch culture was prepared for analysis of short chain fatty acids (SCFA). Sample was centrifuged at 13,680×g for 10 minutes to separate cells and solid particles and supernatant was obtained. After that, sample was filtered through 0.2 µm nylon filter for analysis of acetic, propionic, butyric and lactic with high performance liquid chromatography (HPLC). The HPLC column was BIO-RAD Aminex HPX-87 H Ion Exclusion with diameter 7.8 mm, length 300 mm. Mobile phase was 0.005 M H_2SO_4 with flow rate of 0.6 ml/min temperature at 50°C and UV detector was set at 215 nm (Fernandes *et al*., 2000).

Analysis of vitamins B1, B2 and folic acid

The sample from batch culture was prepared for analysis of vitamin B1, B2 and folic acid. Sample was centrifuged at 13,680×g for 10 minutes to obtain supernatant. After that, the sample was filtered through 0.2 µm nylon membrane. Vitamins were analyzed by HPLC using Inertsil Diol (5 µm) column with diameter 4.6 mm, length 250 mm. Mobile phase was acetonitrile (CH_3CN), water and trifluoroacetic acid (TFA) with ratio as CH_3CN:H_2O:TFA of 90:10:0.1. Flow rate of mobile phase was 1.0 ml/min, column oven was set to 40°C and UV detector at 254 nm was used (Xueyan and Denis 2006).

Results and Discussion

Production of galactooligosaccharide

The highest GOS was produced by the method of Hemmaratchirakul *et al.* (2013). Production of GOS was achieved by using 60% lactose as substrate reacted with β-galactosidase. Approximately 14% of GOS was obtained within 7 hours as compared to other experiments in Table 1. The results showed that transgalactosylation could produce GOS at a high lactose concentration more than at a low lactose concentration. This was because the low lactose condition had been using some water to hydrolyze lactose to glucose and galactose. If there was much water in reaction, the reaction prefers on hydrolysis rather than transgalactosylation. Production of GOS have been studied for about four decades to improve yield and to obtain differ DP and type of linkages. Yield is directly related to cost effectiveness for pilot-scale or commercial scale production. Distribution of DP and type of linkages reflected to prebiotic property or gut fermentation property. Production of GOS have been carried out not only by free enzyme (β-galactosidase) in batch operation. It was also successful performed in UF membrane system using either lactose or cow milk (data not show) similar to the work of Chockchaisawasdee (2004). Recent data showed that GOS can be synthesized by immobilized cells of *Kluyveromyces marxianus* NCIM 3551. However, GOS yield (7.2%) obtained was much lower than in this study (14%) (Srivastava *et al*., 2015). Although extensive researches have been investigated however lactose conversion into β (1–4) GOS never exceeds 50%. Such is the case

Table 1. Comparison of the conditions for production of galactooligosaccharide in this study and other experiments

Enzyme source	Enzyme conc. (U/ml)	Initial lactose conc. (g/L)	Final GOS conc. (%)	Start-up time (h)	Reference
Thermotoga maritima	1.5	500	18	5.0	Ji *et al.* (2005)
Kluyveromyces lactis	2	230	22	4.5	Foda and Lopez (2000)
Kluyveromyces lactis	6	280	22	1.5-2.0	Chockchai sawasdee *et al.* (2004)
Aspergillus oryzae	10,000	400	25	0.5	Albayrak and Yang (2002)
Aspergillus oryzae	42.3	270	22	0.25	Norman (2006)
Lactobacillus pentosus var. *plantarum* BFP32	100	600	14	7.0	This study

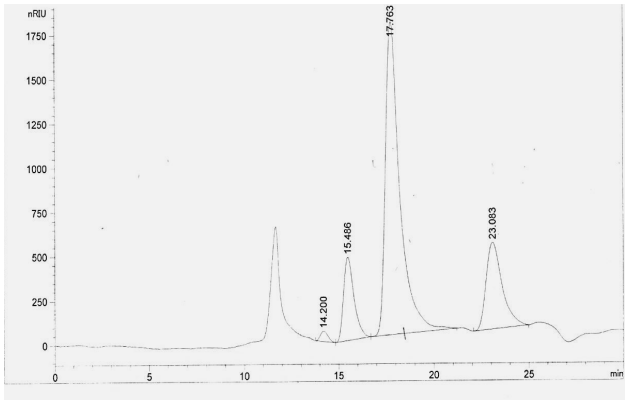

Figure 1. Chromatogram of freeze dried galactooligosaccharide after purified by yeast *Saccharomyces cerevisiae* TISTR5019 fermentation at 30°C for 24 hours

for the β-galactosidase catalyzing the generation of Vivinal GOS® or the process using *Sporobolomyces singularis* membrane bound β-hexosyl transferase to catalyze the synthesis of Oligomate 55N/55NP (Bruno-Barcena and Azcarate-Peril, 2015).

Purification of GOS by Saccharomyces cerevisiae *TISTR5019*

The yeast used in this study for purification of GOS mixture was *S. cerevisiae* TISTR5019. The yeast could metabolize glucose completely and also utilize galactose at 98% of initial concentration in 48 hours. At high concentrations of sugar, *S. cerevisiae* showed more effectiveness on glucose and galactose utilization. The purified GOS was obtained after yeast fermentation then the GOS syrup was further dried by freeze dryer. The chemical composition of dried GOS composed of DP3 and DP4 analyzed by HPLC as showed in Figure 1. It was found that GOS content was about 14% of total sugar. In addition, purification of the GOS mixture was also carried out by nanofiltration using a spiral wound membrane (model DL2540, GE Osmonics Inc.) (Hemmaratchirakul *et al*., 2013). However, it was found that under these conditions it cannot completely fractionate GOS from glucose, lactose and galactose. Hernandez and colleague (2009) reported on fractionation of GOS by various methods but it seems to be not applicable for industrial scale. Recently it has been reported that using a NF membrane (NP030) at 45 bar could obtain GOS purity of 85% (based on monosaccharide content) and an OS recovery yield of 82% (Pruksasri *et al*., 2015). However, GOS was also lost about 30% during filtration with diafiltration similar to our study.

Prebiotic properties

The results from fecal batch cultures of the GOS produced, commercial GOS and mixed sugars (galactose and lactose) were compared in term of prebiotic index (PI). It was found that the GOS produced with 14% of total sugar and commercial GOS with 42% of total sugar had positive value of prebiotic index but mixed sugars had negative value of prebiotic index (Table 2). So that the GOS produced and commercial GOS had prebiotic properties but mixed sugars had no prebiotic properties. Prebiotic index properties could be calculated from prebiotic index formula by using cell numbers from cells counting with fluorescent *in situ* hybridization (FISH) technique. The microbial group that counted as Lactobacilli, Bifidobacteria, Clostridia, Bacteroides and Eubacteria. The result showed that the GOS produced could increase Lactobacilli and decrease clostridia groups significantly ($p<0.05$). Mixed sugars could increase Lactobacilli, and decrease Bifidobacteria groups significantly ($p<0.05$). And commercial GOS could increase Lactobacilli, Bifidobacteria and decrease clostridia groups significantly ($p<0.05$). The numbers of bacterial counts in fecal batch culture are shown in Figure 2.

Table 2. The prebiotic index (PI) of the GOS produced and commercial GOS

Sample	Composition (%)				Prebiotic index
	GOS	Lactose	Glucose	Galactose	
GOS (in this study)	14	61	0	25	1.19
Commercial GOS	49	34	14	3	1.68
Lactose + Galactose	0	61	0	39	-0.01

It had been reported that prebiotic index of GOS has positive value however its values were varied in range of 4.2 (Guerrero *et al*., 2015), 3.5-7.2 for DP3 and 4, respectively (Li *et al*., 2015). Variation of PI value may cause by experimental conditions, calculating equation, molecular weight distribution and type of linkages. Ladirat *et al*. (2014) suggested that GOS, especially its large size-fractions (DP4-7), support the recovery of bifidobacteria and butyrate-producing bacteria whereas low size-fractions (DP2-3) preferred metabolized by non-amoxicillin treated human fecal inoculum.

Short chain fatty acid production

Synthesis of short chain fatty acids during fecal fermentation showed in Table 3. Comparison of the GOS produced, commercial GOS and mixed sugars, it was found that the GOS produced could produce highest in butyric acid. Commercial GOS could produce highest in lactic acid. Mixed sugars could promote growth of lactic acid bacteria thus

Table 3. Short chain fatty acids produced by fecal fermentation of GOS using *in vitro* batch culture

Sample	Time (h)	Lactic acid (µg/ml)	Acetic acid (µg/ml)	Butyric acid (µg/ml)	Propionic acid (µg/ml)
GOS (in this study)	0	0	4.98±1.44	4.73±1.97	2.30±1.62
	3	2.75±1.02	7.95±1.60	42.18±1.49	2.37±0.56
	9	3.20±1.08	13.4±1.45	2.03±1.29	1.76±1.30
	12	3.55±1.16	16.5±1.03	0.98±1.87	1.28±0.93
	24	5.30±1.57	31.63±1.22	ND	ND
Lactose+ Galactose	0	0	3.34±2.13	34.64±0.76	1.13±0.28
	3	2.16±1.05	5.47±1.09	9.35±1.36	1.11±1.09
	9	11.69±1.26	7.53±1.05	1.22±1.35	0.90±1.18
	12	23.11±1.14	10.08±1.06	0.54±1.54	0.86±1.06
	24	5.25±1.00	16.16±1.51	ND	ND
Commercial GOS	0	0	3.06±1.20	42.47±1.29	3.00±1.89
	3	3.41±1.08	5.83±1.07	28.87±1.13	1.78±1.09
	9	18.51±1.05	9.79±1.12	11.36±1.24	1.16±1.14
	12	31.15±1.05	11.02±1.07	2.68±1.29	0.90±1.18
	24	10.57±1.39	18.77±1.72	ND	2.27±1.81

*ND = Not detected

increase in lactic acid. The GOS produced showed sharply increase in acetic acid (31 µg/ml) at 24 hours. Commercial GOS and mixed sugars had gradually increased of acetic acid at similar concentration but it was lower than the GOS produced. For production of butyric acid, it was found that the GOS produced could produce more butyric acid than commercial GOS and mixed sugars. This result showed that the GOS produced could produce highest butyric acid within 3 hours thus it has potential for butyrogenic effect. All samples tested showed that it had low in production of propionic acid although commercial GOS and the GOS produced had slightly higher than mixed sugars. Butyric and propionic acids were good source of energy for colonic cells (Naidu *et al.*, 1999) and it could reduce risk of colon cancer in mammal including human. Cummings and Englyst (1995) found that propionic was source of ATP in liver and could reduce cholesterol in human blood. Butyric acid used in cell division in colon and it as cellular proliferation and program cell death control (apoptosis). Recent reviews suggested that GOS could be used for lowering risk of colorectal cancer by supporting on growth of specifically the probiome (autochtonous beneficial bacteria) (Bruno-Barcena and Azcarate-Peril, 2015).

Vitamins production

Production of vitamins in fecal batch culture showed that the GOS produced could promote the growth of microbial to produce vitamin B1 and B2 about 9 hours and after that vitamin B2 sharply decreased while vitamin B1 slightly increased at 24 hours. Folic acid could be produced by colonic microbiota in first 3 hours of fecal fermentation and decreased thereafter. Commercial GOS could promote vitamin B1 production but vitamin B2 and folic acid were not produced. It was similar to mixed sugars that do not show prebiotic property and not produce any vitamin. The fluctuation in changes of SCFA and vitamins production may cause by it is produced by some species and later on it is utilized by other species simultaneously.

Conclusions

Conditions for enzymatic synthesis of GOS were 60% (w/v) lactose as substrate reacted with 100 U/ml β-galactosidase, at 50°C and pH 7.0. Purification of GOS mixture by *Saccharomyces cerevisiae* TISTR 5019 had the highest efficiency to remove glucose. The purified GOS syrup was freeze dried and chemical composition was analyzed by HPLC. GOS powder contained 14% GOS with DP of 3 and

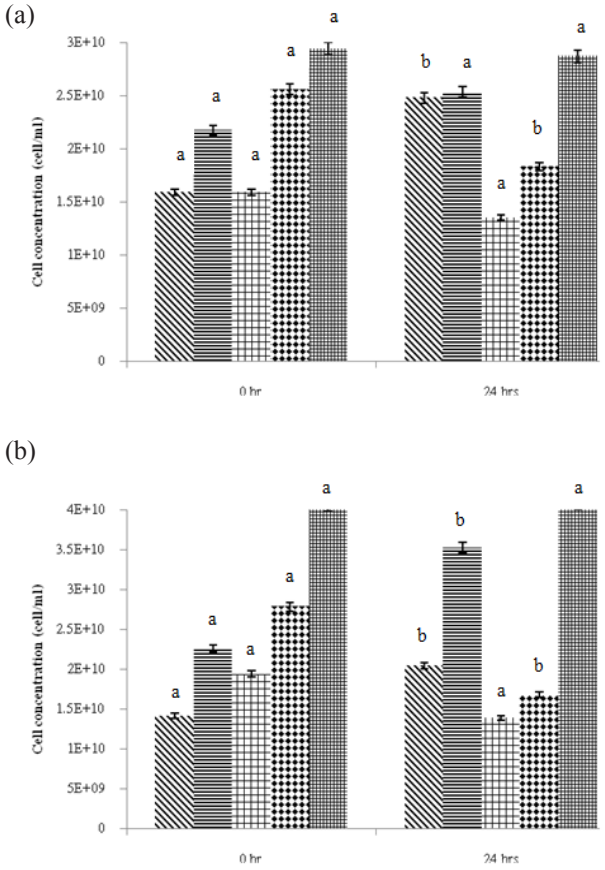

Figure 2. Bacterial counts of (a) the GOS produced, (b) commercial GOS in fecal batch culture at 0 and 24 hours; (▒) Lactobacilli; (▒) Bifidobacteria; (▒) Bacteroides; (▒) Clostridia; (▒) Eubacteria
Different letters mean statistically significant ($p<0.05$)

4 whereas DP3 is a main component. The prebiotic property of the GOS produced was evaluated by in vitro fecal batch culture. It was found that the GOS produced had prebiotic property with PI value of 1.19 whereas commercial GOS and mixed sugars (non-prebiotic) had PI of 1.68 and -0.01, respectively. There was also SCFA produced particular butyric acid and vitamins B1, B2 but folic acid could not be produced. Interestingly, GOS in this study produced about twice higher in butyric acid compared to commercial GOS. Butyric acid is proven that it is related in lowering risk of colon cancer. So that GOS in this study has prebiotic properties comparable to commercial GOS and it has also butyrogenic effect.

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References

Albayrak, N. and Yang, S. T. 2002. Production of galacto-oligosaccharides from lactose by *Aspergillus oryzae* β-galactosidase immobilized on cotton cloth. Biotechnology Bioengineering 77(1): 8-19.

Bruno-Barcena, J. M. and Azcarate-Peril. M. A. 2015. Galacto-oligosaccharides and colorectal cancer: feeding our intestinal probiome. Journal of Functional Foods 12: 92–108.

Calame, W, Weseler, A. R. and Viebke, C. 2008. Gum arabic establishes prebiotic functionality in healthy human volunteers in a dose-dependent manner. British Journal of Nutrition 9: 1-7.

Chockchaisawasdee, S., Athanasopoulos, V. I., Niranjan, K. and Rastall, R. A. 2004. Synthesis of galacto-oligosaccharide from lactose using beta-galactosidase from *Kluyveromyces lactis*: studies on batch and continuous UF membrane-fitted bioreactor. Biotechnology and Bioengineering 89: 434-443.

Cummings, J. H. 1981. Cellulose and the human gut. Gut 25: 805–810.

Cummings, J. H. and Englyst, H. N. 1995. Gastrointestinal effects of food carbohydrate. The American Journal of Clinical Nutrition 61: 938-945.

Egert, M., De Graaf, A. A., Smidt, H., de Vos, W. M. and Venema, K. 2006. Beyond diversity: functional microbiomics of the human colon. Trends in Microbiology 14: 86-91.

Ekhart, P. F. and Timmermans, E. 1996. Techniques for the production of transgalactosylated oligosaccharides (TOS). Bulletin FIL IDF 313: 59–64.

Fastinger, N. D., Karr-Lilienthal, L. K. and Spears, J. K. 2008. A novel resistant maltodextrin alters gastrointestinal tolerance factors, fecal characteristics and fecal microbiota in healthy adult humans. Journal of the American College of Nutrition 27: 356-366.

Fernandes, J, Rao, A. V. and Wolever, T, M. S. 2000. Different substrates and methane producing status affect short-chain fatty acid profiles produced by in vitro fermentation of human feces. The Journal of Nutrition 30: 1932-1936.

Foda, M. I. and Lopez, L. M. 2000. Continuous production of oligosaccharide from whey using a membrane reactor. Process Biochemistry 35: 581-587.

Gibson, G. R. and Roberfroid, M. B. 1995. Dietary modulation of the human colonic microbiota: introducing the concept of prebiotics. Journal of Nutrition 125: 1401-1412.

Gibson, G. R., Probert, H. M., Loo, V. J, Rastall, R. A. and Roberfroid, M. B. 2004. Dietary modulation of the human colonic microbiota: updating the concept of prebiotics. Nutrition Research Reviews 17: 259–275.

Hemmaratchirakul, J, Wichienchot, S, Jaturapiree, P. and Prueksasri, S. 2013. Production of galacto-oligosaccharides by β-galactosidase from *Lactobacillus* sp. BFP32 and *in vitro* fermentation by human fecal microflora. Songkhla, Thailand: Prince of Songkhla University, MSc thesis.

Hernandez, O., Muiz-Matute, A. I., Olano, A., Moreno, F.

J. and Sanz M. L. 2009. Comparison of fractionation techniques to obtain prebiotic galactooligosaccharides. International Dairy Journal 19: 531-536.

Ji, E. S., Park, N. H. and Oh, D. K. 2005. Galactooligosaccharide production by a thermostable recombinant beta-galactosidase from Thermotoga maritima. World of Journal of Microbiology and Biotechnology 89: 434-443.

Ladirat, S. E. Schols, H. A. Nauta, A., Schoterman, M. H. C., Schuren, F. H. J. and Gruppen, H. 2014. In vitro fermentation of galacto-oligosaccharides and its specific size-fractions using non-treated and amoxicillin-treated human inoculum. Bioactive Carbohydrates and Dietary Fibre 3: 59–70.

Lewis, S. and Atkinson, C. 2009. Designing trials for testing the efficacy of pre- pro- and synbiotics. In Charalampopoulos, D. and Rastall, R. A. (Eds). Prebiotics and Probiotics Science and Technology, p. 111-134. New York: Springer.

Li, W., Wang, K., Sun, Y., Ye, H. Hu, B. and Zeng, X. 2015. Influences of structures of galactooligosaccharides and fructooligosaccharides on the fermentation in vitro by human intestinal microbiota. Journal of Functional Foods 13: 158–168.

Macfarlane, G. T., Macfarlane, S. and Gibson, G. R. 1998. Validation of a three-stage compound continuous culture system for investigating the effect of retention time on the ecology and metabolism of bacteria in the human colon. Microbial Ecology 35: 180-187.

Mountzouris, K. C., Gilmour, S. G., Jay, A. J. and Rastall, R. A. 1999. A study of dextran production from maltodextrin by cell suspensions of *Gluconobacter oxydans* NCIB 4943. Journal of Applied Microbiology 87: 546-556.

Naidu, A. S., Bidlack, W. R. and Clemens, R. A. 1999. Probiotic spectra of lactic acid bacteria (LAB). Critical Reviews in Food Science and Nutrition 38(1): 13-126.

Norman, J. M. 2006. Production, fractionation and purification of galacto-oligosaccharides from whey lactose. Michigan, USA: Michigan University, PhD thesis.

Osman, A. 2015. Synthesis of prebiotic galacto-oligosaccharides: science and technology. In Watson R. R. and Preedy, V. R. (Eds.). Probiotics, Prebiotics, and Synbiotics: Bioactive Foods in Health Promotion, p. 135-154. London: Academic Press.

Otieno, D. O. 2010. Synthesis of β-galactooligosaccharides from lactose using microbial β-galactosidases. Comprehensive Reviews in Food Science and Food Safety 9: 471–482.

Palframan, R. J., Gibson, G. R. and Rastall, R. A. 2003. Development of a quantitative tool for the comparison of the prebiotic effect of dietary oligosaccharides. Letters in Applied Microbiology 37: 281-284.

Pruksasri, S., Nguyen, T. H., Haltrich, D. and Novalin, S. 2015. Fractionation of a galacto-oligosaccharides solution at low and high temperature using nanofiltration. Separation and Purification Technology 151: 124–130.

Roberfroid, M. 2007. Prebiotics: the concept revisited. The Journal of Nutrition Effects of Probiotics and Prebiotics 137: 831S-837S.

Rycroft, C. E., Jones, M. R., Gibson, G. R. and Rastall, R. A. 2001. Fermentation properties of gentiooligosaccharides. Letters in Applied Microbiology 32: 156-161.

Shoaf, K., Mulvey, G. L., Armstrong, G. D. and Hutkins, R. W. 2006. Prebiotic galactooligosaccharides reduce adherence of enteropathogenic *Escherichia coli* to tissue culture cells. Infection and Immunity 74: 6920–6928.

Srivastava, A. Mishra, S. and Chand, S. 2015. Synthesis of galacto-oligosaccharides from lactose using immobilized cells of *Kluyveromyces marxianus* NCIM 3551. Journal of Molecular Catalysis B: Enzymatic 123: 147–153.

Tuohy, K. M., Rouzaud, G. C. M., Bruck, W. M. and Gibson, G. R. 2005. Modulation of the human gut microflora towards improved health using prebiotics—assessment of efficacy. Current Pharmaceutical Design 11: 75–90.

Vernazza, C. L. Rabiu, B. A. and Gibson, G. R. 2006. Human colonic microbiology and the role of dietary intervention: introduction to prebiotics. In Gibson, G. R. and Rastall, R. A. (Eds.). Prebiotics: Development and Application, p. 1-28. Chichester: John Wiley & Sons Ltd.

Wichienchot, S. and Chinachoti, P. 2011. Prebiotic oligosaccharides: origins and production, health benefits and commercial applications. In Gordon, N. S. (Ed). Oligosaccharides: Sources, Properties and Applications, p. 59-83. New York: Nova Science Publishers, Inc.

Wichienchot, S., Jatupornpipat, M. and Rastall, R. A. 2010. Oligosaccharides of pitaya (dragon fruit) flesh and their prebiotic properties. Food Chemistry 120(3): 850-857.

Xueyan, T. and Denis, A. C. 2006. A simplified approach to the determination of thiamine and riboflavin in meats using reverse phase HPLC. Journal of Food Composition and Analysis 19: 831-837.