International Food Research Journal 22(6): 2550-2557 (2015)
Journal homepage: *http://www.ifrj.upm.edu.my*

Production of galactooligosaccharide by β-galactosidase from *Lactobacillus pentosus* var. *plantarum* BFP32

[1]Hemmaratchirakul, J., [2]Jaturapiree, P., [2]Prueksasri, S. and [1*]Wichienchot, S.

[1]*Interdisciplinary Graduate School of Nutraceutical and Functional Food, The Excellent Research Laboratory for Cancer Molecular Biology, Prince of Songkla University, Hat Yai, Songkhla, Thailand 90112*
[2]*Department of Biotechnology, Faculty of Engineering and Industrial Technology, Silpakorn University, Nakorn Pathom, Thailand 73000*

Article history

Received: 27 October 2014
Received in revised form: 4 May 2015
Accepted: 20 May 2015

Keywords

Galactooligosaccharide
β-galactosidase
Lactobacillus pentosus
Nanofiltration
Prebiotic

Abstract

Among 52 isolates lactic acid bacteria, BFP32 showed highest intracellular β-galactosidase activity and galactooligosaccharide (GOS) yield. It was identified as *Lactobacillus pentosus* var. *plantarum* by 16S rDNA sequencing. The K_m values of the β-galactosidase using oNPG and lactose as substrate were 40.00 and 33.33 mM, respectively. The V_{max} values were 2.198 and 76.923 mM min^{-1}, respectively. The optimum reaction temperature and pH for producing GOS from 10% lactose were 50°C and 7.0. A maximum GOS yield (13.90%) was obtained using 60% lactose as substrate. Composition of GOS consisted of oligosaccharides with having several degree of polymerization (DP) of 3, 4 and 5. A mixture of GOS was purified successful by sequential bacterial and yeast fermentation whereas nanofiltration could be used for partial purification. The β-galactosidase from *L. pentosus* var. *plantarum* BFP32 was intracellular β-galactosidase and it showed transgalactosylation activity at concentration of lactose higher than 10% for production of GOS.

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 (Buddington, 2009). The benefits of prebiotics have been proven as anti-colon cancer, anti-pathogens in gastrointestinal tract, giving increased calcium adsorption, and improved microbial balance in the gut (Vernazza *et al*., 2006; Wichienchot and Chinachoti, 2011).

GOS are composed of a chain of 2–9 galactose units and a terminal glucose. GOS are considered prebiotic compounds since they pass undigested through the small intestine in humans to reach the colon (Chen *et al*., 2002; Sangwan *et al*., 2011).

In the present work, the sources of β-galactosidase from various fermented foods and non-food samples were explored. The bacterial isolate with high β-galactosidase activity was identified, and the kinetics of the β-galactosidase were calculated. The optimal conditions for GOS synthesis from lactose were studied. The chemical compositions and degree of polymerization of the GOS produced were investigated. This work aimed to screen bacterial producing β-galactosidase from several Thai fermented foods, breast milk and infant feces, bacterial identification, kinetics of the enzyme, optimal conditions for production of GOS and purification by bacterial and yeast fermentation compared with nanofiltration.

Materials and Methods

Samples and chemicals

Food samples (fermented pork, sour sausage, fermented fish, and pickle) were purchased from a local market in Bangkok and Nakornphatom, Thailand. Non-food samples (breast milk and infant feces) were collected from healthy volunteers in Nakornphatom, Thailand. Unless otherwise stated, all chemical reagents and enzymes were obtained from Sigma-Aldrich Co. Ltd. Culture media were supplied by Merck, Co., Ltd., Germany.

Screening and isolation of lactobacilli producing β-galactosidase

One gram of homogenized samples (fermented foods, feces, breast milk) was placed in 0.1% peptone, and incubated at 37°C for 24 hours. Then the sample

*Corresponding author.
Email: *santad.w@psu.ac.th*
Tel: +66 74289494

was diluted with MRS broth to obtain serial dilutions from 10^{-3} to 10^{-6}. One milliliter of each dilution was poured on to MRS + bromocresol green agar then incubated at 37°C for 24 hours. After screening of lactobacilli producing β-galactosidase by cultivation on MRS + X-gal + IPTG agar, the colonies that changed from white to blue colonies were selected.

Selection of the lactobacilli that have the highest enzyme activity

The selected strains were cultured in MRS broth at 37°C for 16 hours for preparing crude enzyme. Then the sample was centrifuged (11,400×g) at 4°C for 15 minutes. The pellet was washed twice with 50 mM phosphate buffer (pH 7.0) followed by z-buffer and acetone:toluene (9:1) was added and mixed for 5 seconds. Incubation was carried out at 37°C for 20 minutes.

Enzyme activity was determined using 450 µl of 22 mM oNPG with 50 µl of the β-galactosidase incubated at 37°C for 15 minutes. And the reaction was terminated by 750 µl of Na_2CO_3. Enzyme activity was measured by spectrophotometer at 420 nm and compared with the z-buffer instead of enzyme. One unit of β-galactosidase is defined as enzyme activity that delivered 1 molecule of galactose and glucose from lactose within 1 second. The enzyme activity was calculated by the following formula:

$$\text{Activity (U/ml)} = \frac{OD420}{0.6854} \times \frac{480+20}{20} \times \frac{750+500}{500} \times \frac{1}{\text{min}} \times \text{dilution factor}$$

Identification of the lactobacilli

Pure bacterial culture (isolate BFP32) with a single colony on MRS agar was identified by a certified laboratory of BIOTEC Culture Collection (BCC), National Center for Genetic Engineering and Biotechnology, Thailand. The method of identification was 16S rDNA sequencing comprising of three steps. First step was PCR amplification of 16S rDNA (Kawasaki *et al.*, 1993; Yamada *et al.*, 2000; Katsura *et al*., 2001). Second step was sequencing of the purified PCR product (Brosius *et al*., 1981). Third step was sequence analyses using Cap contig assembly program. The identification of phylogenetic neighbors was initially carried out by the BLAST (Altschul *et al.*, 1997) and megaBLAST (Zhang *et al.*, 2000; Chun *et al*., 2007).

Characterization of the β-galactosidase on oNPG

Effect of temperature

The reaction mixture consisted of 450 µl of 22 mM oNPG in 50 mM phosphate buffer (pH 7.0) with an addition of 50 µl crude enzyme from *L. pentosus* var. *plantarum* BFP32. The reaction was incubated by shaking in a water bath at various temperatures (37, 40, 45 and 55°C) for 15 minutes. The reaction was terminated by adding 750 µl of Na_2CO_3 and sample was measured by spectrophotometer at 420 nm. The enzyme activity and percentage of relative activity were calculated.

Effect of pH

The effect of the pH on the β-galactosidase activity was assessed when the reaction mixture consisted of 450 µl of 22 mM oNPG in 50 mM phosphate buffer with various pH levels (5.0, 5.5, 6.0, 6.5, 7.0, 7.5 and 8.0) by addition of 50 µl crude enzyme from *L. pentosus* var. *plantarum* BFP32. The reaction was incubated by shaking in a water bath at 40°C for 15 minutes, after the reaction was terminated, it was measured by spectrophotometer at 420 nm. Then the enzyme activity and percentage of relative activity were calculated.

Enzyme kinetic on oNPG

A kinetic study of the β-galactosidase on oNPG was carried out to determine the K_m and V_{max} values under optimal conditions. In the case of using oNPG as a substrate, the reaction mixture consisted of 10 ml oNPG at various concentrations (2, 4, 6, 8, 10, 15, 20, and 22 mM) in 50 mM phosphate buffer (pH 7.0). Then 10 ml of crude enzyme from *L. pentosus* var. *plantarum* BFP32 was added to obtain the final enzyme concentration of 5 U/ml. The reaction was incubated by shaking in a water bath at 40°C for 15 minutes, and the reaction was terminated with 750 µl of Na_2CO_3. Sample was measured by spectrophotometer at 420 nm. The Lineweaver-Burk was plotted and K_m and V_{max} were calculated.

Characterization of the β-galactosidase on lactose

Effect of temperature

Ten ml of 5.0% lactose in 50 mM phosphate buffer (pH 7.0) was added to 10 ml of crude enzyme from *L. pentosus* var. *plantarum* BFP32 to obtain the final enzyme concentration of 5 U/ml. The reaction mixture was incubated by shaking in a water bath at various temperatures (37, 40, 45, 50, 55, 60, 65, and 70°C) for 48 hours. The enzyme was inactivated by heating at 95°C for 15 minutes and the sugar profiles, including the GOS were measured by HPLC.

Effect of pH

Ten ml of 5.0% lactose in 50 mM phosphate buffer at various pH levels (6.0, 6.5, 7.0, 7.5 and 8.0) was added to 10 ml crude enzyme from *L. pentosus*

var. *plantarum* BFP32 to obtain final enzyme concentration of 5 U/ml. The reaction mixture was incubated by shaking in a water bath at 50°C for 48 hours. The enzyme was inactivated by heating at 95°C for 15 minutes and the sugar and GOS were analyzed by HPLC.

Enzyme kinetics on lactose

For lactose used as substrate, the reaction mixture consisted of 10 ml lactose at various concentrations (2.5, 5.0, 7.5, 10.0, 12.5, 15.0, 17.5, and 20.0%) in 50 mM phosphate buffer (pH 7.0) were prepared. The final enzyme concentration at 5 U/ml was obtained. The reaction mixture was incubated by shaking in a water bath at 50°C for 48 hours, and the enzyme was inactivated by heating at 95°C for 15 minutes. The sugars and GOS were analyzed by HPLC.

Determination of sugar composition and degree of polymerization

Mono-, di- and oligosaccharide were analyzed by HPLC. A sample was filtered through a 0.2 µm membrane and 5 µl samples were injected into 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 was 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 sugar. 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 after removal of the low molecular weight sugars by nanofiltration or bacterial and yeast fermentation was determined by comparing the sample retention time 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 various concentrations (20%, 40% and 60%) was used as a substrate for producing galactooligosaccharide with optimum temperature and pH. The reaction was performed for 48 hours and then it was terminated by heating at 95°C for 15 minutes. The analysis of sugar content and GOS was done by HPLC as mentioned above.

Purification of galactooligosaccharide

Fractionation by ultra- and nanofiltration

The method for purification was done using diafiltration technique on a membrane reactor. The ultrafiltration; KERASEPTM, tubular ceramic membrane, 19 channels with 3.5 mm diameter, surface area per tube was 0.245 m^2. Diameter/length was 25 mm/1178 mm, 50 kDa MWCO, transmembrane pressure was 5 bars and flow rate was 2000 l/h. It was used for filtering some contamination in first time and nanofiltration; model DK2540 (GE Osmonics Inc.), spiral wound type, active area was 1.77 m^2, diameter/length was 2.5"/40", 300 kDa MWCO, transmembrane pressure was 10 bars and flow rate was 500 l/h. This membrane was used for removal of monosaccharide and disaccharide. Filtration was performed until the volume of retentate was a half of initial volume. Retentate was then added by water of a half to get initial volume for diafiltration. This diafiltration was performed for 3 times consecutively then sugar content in retentate and permeate at each diafiltration step were determined by HPLC.

Purification by bacterial and yeast fermentation

The microorganism for purification of GOS mixture was *Lactobacillus pentosus* var. *plantarum* BFP32 for elimination of disaccharide (lactose). This bacterium could metabolize lactose to produce lactic acid that could decrease pH to 6.5-6.8. This pH was optimum condition for growth of *Saccharomyces cerevesiae* TISTR5019 for elimination of monosaccharide (glucose and galactose). *S. cerevisiase* could metabolize glucose to produce alcohol and it could utilize galactose by the enzymes of Leloir pathway. 5% of *L. pentosus* var. *plantarum* BFP32 was used as inoculum and it was incubated for 12-48 hours in anaerobic condition. Reaction was terminated by heating at 95°C for 15 minutes. Fermentation of GOS mixture was carried out in a rotary shaker at 180 rpm for 12-24 hours, reaction was terminated and alcohol was evaporated by heating at 95°C for 15 minutes. Sample was taken for analysis of sugar content and GOS by HPLC.

Results and Discussion

Screening and isolation of lactic acid bacteria producing β-galactosidase

Lactic acid bacteria (LAB) were screened in fermented foods and non-food samples in Thailand. The method of screening was based on acid producible microorganism by changing the color of MRS+Bromocresol green agar from green to yellow.

It was found that 77 isolates could produce acid; moreover, 77 isolates were further screened for the production of β-galactosidase by cultivation of the isolates on MRS+IPTG+X-gal agar. It was found that 52 isolates from 77 had β-galactosidase activity as the color of the colonies changed from white to blue. Among the 52 isolates producing β-galactosidase, the top five isolates with the highest β-galactosidase activity were BFP32, BFP20, BFP15, CFP07 and BFP01, respectively. Surprisingly, most of them were isolated from fermented pork. This is the first report reveals that LAB in fermented pork produced β-galactosidase. However some β-galactosidase producers were isolated from fermented foods such as dairy products. The maximum GOS yields of the isolates were 1.01, 0.97, 0.87, 0.85 and 0.77 (% total sugar) for BFP32, BFP20, BFP15, BFP19 and BFP24, respectively. The isolate BFP32 was chosen for extraction of intracellular β-galactosidase. Since this isolate gave the highest GOS yield by conversion of 5% lactose to 1.01% GOS within 4 hours, 5 U/ml β-galactosidase at 50°C, pH 7.0.

Identification of the lactobacilli

The isolate BFP32 gave the highest β-galactosidase activity and it was also gave the highest GOS yield produced from lactose. Therefore the BFP32 isolate was further identified by 16s rDNA sequencing. This isolate was identified as *Lactobacillus pentosus* var. *plantarum* since their sequence showed the highest pairwise similarity of 99.931 and 99.862% for *L. pentosus* and *L. plantarum*, respectively. The phylogenetic tree of BFP32 and similar bacteria was constructed as shown in Figure 1. The sequence of BFP32 was confirmed as closest to *L. pentosus* and *L. plantarum* by gene bank and it was provided an accession number of JQ288726. The genetics of BFP32 were closest to *Lactobacillus pentosus* JCM 1558 (accession no. D79211) and *Lactobacillus plantarum* subsp. *plantarum* ATCC 14917 (accession no. ACGZ01000098). This is a new strain for production of GOS. However only *Lactobacillus reuteri* had been reported and the optimal conditions for the strain were 20.5% lactose, 37°C, pH 6.5, 80% lactose conversion, giving a maximum GOS yield of 38% (Splechtna *et al*., 2006). *L. pentosus* var. *plantarum* BFP32 is safe for use in food products since it has been defined as a probiotic strain.

Characterization of the β-galactosidase on oNPG

Effect of temperatures

The activity of enzyme was significantly depended on the temperature of the reactions. The highest β-galactosidase activity was obtained at 40°C and the activity was sharply decreased at lower temperatures. When the reaction temperature increased to over 40°C, the enzyme activity showed a gradually decrease (Figure 2a). The percentage of relative activity at 37, 40, 45 and 55°C were 78.08, 100.00, 87.67 and 58.90, respectively. The optimum temperature of the β-galactosidase activity seemed to be largely depended on the sources from where the microorganism was isolated. The optimum temperature of BFP32 β-galactosidase on oNPG was 40°C whereas the β-galactosidases for producing commercial GOS were mostly thermophilic bacteria.

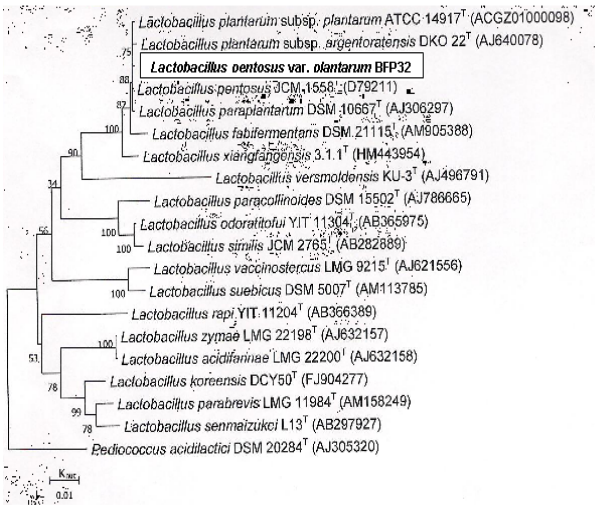

Figure 1. Phylogenetic tree of *Lactobacillus pentosus* var. *plantarum* BFP32 isolated from fermented food compared to relative similar species

Effect of pH

The enzyme activity depended on reaction pH. The highest β-galactosidase activity was obtained at pH 7.0 and at a lower pH the activity was slightly decreased (Figure 2b). The percentage of relative activity at pH 5.0, 5.5, 6.0, 6.5, 7.0, 7.5 and 8.0 were 81.16, 85.51, 88.41, 94.20, 100.00, 81.16 and 69.57, respectively. Typically, the optimum pH of β-galactosidase for the commercial production of GOS is preferably an acidic condition. An optimum pH of the BFP32 β-galactosidase on oNPG was 7.0 which was the same pH value of β-galactosidases from *Kluyveromyces lactis* using lactose as a substrate (Chockchaisawasdee *et al*., 2005).

Enzyme kinetics on oNPG

The kinetics of the enzyme were determined at various oNPG concentrations reacting with 5 U/ml final concentration of the BFP32 β-galactosidase. The Lineweaver-Burk plot showed that K_m and V_{max} of the β-galactosidase were 40.00 mM and 2.198 mM/min, respectively. This enzyme had probably less activity

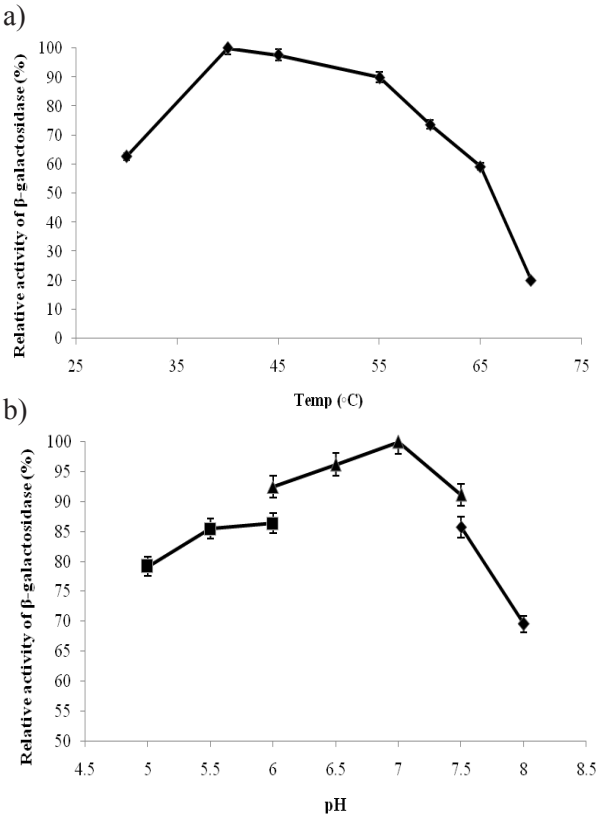

Figure 2. Relative activity of β-galactosidase at various reaction temperatures (a) and pH (b) using oNPG as substrate

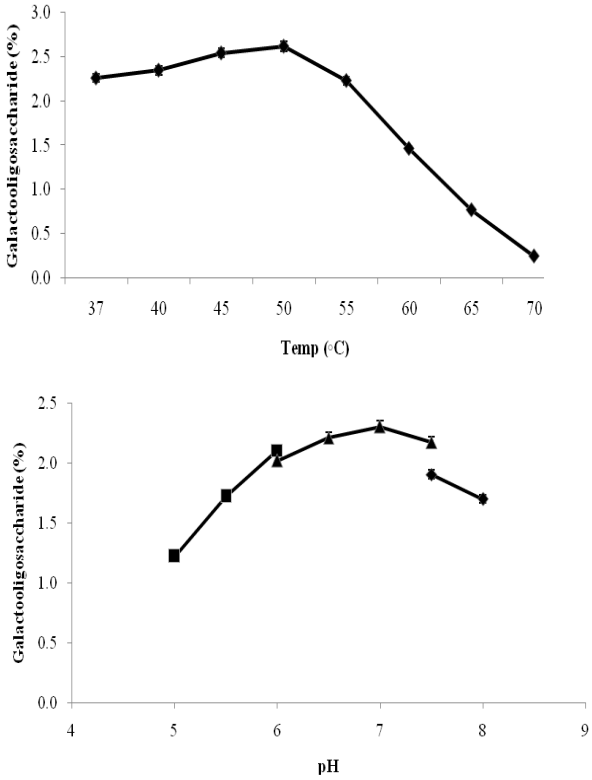

Figure 3. Galactooligosaccharide production at various temperatures (a) and pH (b) using 10% lactose as substrate

due to it was used in form of crude enzyme. Further purification process of the crude enzyme is suggested to obtain higher activity of the enzyme.

Characterization of the β-galactosidase on lactose

Effect of temperatures

The catalytic reaction of the enzyme on lactose, galactose and glucose depended on the reaction temperature. It was found that BFP32 β-galactosidase had the highest hydrolysis activity at 50°C using low concentration of lactose. When raising the reaction temperature to higher than 50°C, the GOS could not be synthesized even at high lactose concentration. This is because of at 60°C and higher the enzyme seemed to be inactivated. The optimum temperature for the production of GOS from lactose was 50°C and gave a yield of 2.7% (Figure 3a).

Effect of pH

The catalytic reaction of the enzyme on lactose to galactose and glucose also depended on pH of the reaction. It was found that BFP32 β-galactosidase gave the highest hydrolysis activity at pH 7.0 and low lactose concentration. When pH of the reaction was raised from 7.0 to 8.0 or decreased from 7.0 to 6.0 the lactose concentration was higher. The GOS could not be synthesized because the enzyme could not transfer free galactose moiety to acceptor molecule. At a pH of 7.0-7.5, galactosyltransferase activity was highest since it gave the highest yield of GOS. The optimum pH for the production of GOS from lactose was 7.0-7.5 and it gave a yield of 2.3% (Figure 3b).

Enzyme kinetics and lactose

The kinetics of the enzyme was determined as the various lactose concentrations reacted with 5 U/ml BFP32 β-galactosidase. The Line-waver Burk plot showed that the mM and mM/min of the β-galactosidase were 33.33 mM and 76.92 mM/min, respectively. This enzyme favored lactose more than oNPG as a substrate for production of GOS. However, the kinetics of the enzyme indicated that the enzyme had less efficiency on GOS production from lactose compare to the commercial enzyme.

Production of galactooligosaccharide

The highest GOS (15% of total sugar) was produced by using 60% lactose as a substrate in 8 hours (Figure 4). Forty percent and 20% of lactose as substrate could produce GOS of 10% and 3%, respectively. The results show that transgalactosylation favored to produce GOS at a high lactose concentration more than at a low lactose concentration. This is because the low lactose condition had been using some water

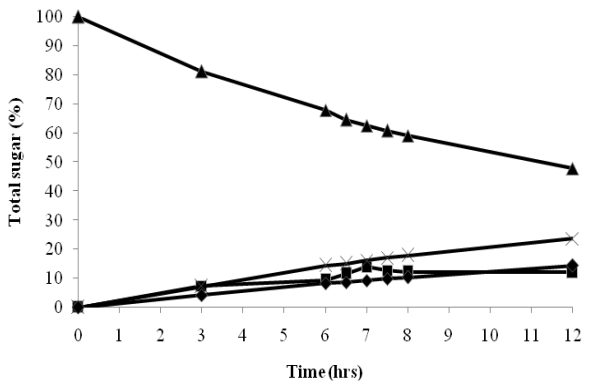

Figure 4. GOS, lactose, glucose and galactose formation by β-galactosidase (100 U/ml) with 60% lactose as substrate at 50°C and pH 7.0; (■) GOS; (▲) lactose; (X) glucose; (◆) galactose

to hydrolyze lactose to glucose and galactose. If there is much water in reaction, the reaction favors on hydrolysis rather than transgalactosylation. Galactooligosaccharide yield is highly depends on microbial sources of β-galactosidase and production conditions. A summary of previous reports showed that GOS yields are in range of 18-52.5% depends on bacterial sources and production conditions (Gosling *et al.*, 2010). In commercial production of GOS, mostly β-galactosidase is derived from *Kluyveromyces lactis* and *Aspergillus oryzae* using 25-40% lactose as substrate (Foda and Lopez-Leiva, 2000; Albayrak and Yang, 2002). However, only two probiotics *L. reuteri* (Splechtna *et al.*, 2006) and *Bif. longum* (Hsu *et al*., 2005) have been reported for production of GOS. In addition, *L. pentosus* var. *plantarum* BFP32 is a new probiotic strain for production of GOS and they could synthesize GOS at very high lactose concentration of up to 60%.

In addition, the study (data not show) was also revealed that milk could be used as alternative substrate for production of GOS in a membrane reactor in one step. This finding has been patented. The conclusion could be drawn that β-galactosidase from *L. pentosus* var. *plantarum* BFP32 is capable to produce GOS using either lactose or milk as a substrate.

Purification of galactooligosaccharide

Fractionation of galactooligosaccharide by UF and NF filtration

Galactooligosaccharide was purified by fractionation using the ultra- and nanofiltration membrane coupled with diafiltration technique. It was found that the optimal diafiltration volume of water was 3. It was found that the residual sugars in permeate were 12% GOS, 14% lactose, 16.6%

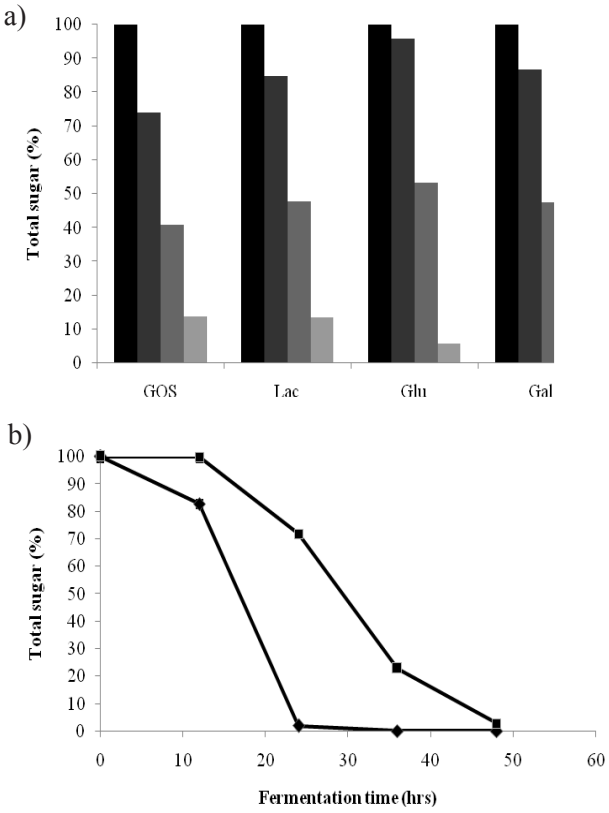

Figure 5. GOS and sugars profiles in retentate after GOS mixture was purified by; (a) nanofiltration with 300 kDa MWCO membrane at various diafiltration cycles, (■) before filtration, (■) 1st diafiltration, (■) 2nd diafiltration, (■) 3rd diafiltration; (b) yeast *Saccharomyces cerevesiae* TISTR5019 fermentation for 48 hrs, (▲) glucose and (■) galactose

glucose and 15% galactose. The residual sugars in the retentate were 13% GOS, 13.5% lactose, 5% glucose and 10% galactose (Figure 5a). This purification method was successful for the removal of undesirable sugars (disaccharide and monosaccharide). However some of GOS was slightly decreased in the retentate. Goulas *et al.* (2002) reported that purification of commercial GOS mixture by nanofiltration using NF-CA-50 membranes and DS-5-DL membranes, gave yield values of 14–18% for the monosaccharide, 59–89% for the disaccharide and 81–98% for the trisaccharide (oligosaccharide), respectively. They also reported that nanofiltration with diafiltration of oligosaccharide from mixtures containing contaminant monosaccharides gave similar results to this study. Although nanofiltration was performed with diafiltration but this purification method seems to be less effective for separation of oligosaccharides from mono- and di-saccharides (Hernandez *et al*., 2009).

Purification of GOS by using bacterial and yeast fermentation

The microorganisms used for purification of

GOS mixture in the study were bacterium *L. pentosus* var. *plantarum* BFP32 and yeast *S. cereviciase* TISTR5019. *L. pentosus* var. *plantarum* BFP32 could utilize glucose as a carbon source at concentrations range from 20% to 40%. Moreover, glucose and galactose were used for 50% and 30%, respectively within 48 hours. But lactose at higher than 40%, *L. pentosus* var. *plantarum* BFP32 showed less utilization of lactose because the high concentration of sugar gave higher osmotic pressure leading to stress to the cells and a decrease in their growth. *S. cerevisiase* TISTR5019 could metabolize glucose completely and also utilize galactose at 98% of initial concentration within 48 hours (Figure 5b). At high concentration of sugar, *S. cerevisiase* TISTR5019 showed more effectiveness on glucose and galactose removal from GOS mixture. Hernandez *et al*. (2009) reported that among four fractionation techniques (diafiltration, yeast treatment, activated charcoal treatment and ethanol precipitation), it was found that yeast treatment is the most effective for removal of monosaccharides. This study was also confirmed this conclusion. However, to get the purest GOS fractions for analysis purpose could be obtained using size exclusion chromatography. This study showed that removal of mono- and di-saccharides from mixture of GOS by sequential fermentation with lactic acid bacteria followed by yeast fermentation has potential for applicable in pilot-scale.

Conclusion

Fermented foods would be a good source for the isolation of lactic acid bacteria. 77 isolates were capable of producing acid, whereas 52 bacterial isolates were capable of producing β-galactosidase. Isolate BFP32 produced the highest GOS within 4 hours at 5 U/ml of enzyme. This isolate was identified as *Lactobacillus pentosus* var. *plantarum* (gen bank accession number JQ288726). This is a new probiotic strain for the production of GOS. The K_m values of the β-galactosidase using oNPG and lactose as substrate were 40.00 and 33.33 mM, respectively. The V_{max} values were 2.198 and 76.923 mM min^{-1}, respectively. The optimum reaction temperatures for the production of GOS from 10% lactose was 50°C whereas the optimum lactose pH was 7.0. The maximum GOS yield using 60% lactose under optimal conditions was 13.90%. The β-galactosidase from isolated strain need to be further purified to obtain higher transgalactosidase activity. The maximum GOS yield could be raised by increasing the β-galactosidase activity. The removal of residual lactose from GOS mixture was successful by *L. pentosus* var. *plantarum* BFP32 converted to glucose and galactose then they were further utilized by *S. cerevisiase* TISTR5019. However, the removal of sugars by nanofiltration with diafiltration in a membrane reactor has some limitations. The β-galactosidase produced by *L. pentosus* var. *plantarum* BFP32 could synthesize GOS from lactose at a very high concentration of 60% (w/w). This finding could be applicable for producing synbiotic dairy products containing prebiotic GOS and probiotic in one step. It is noted that this finding has been patented for applications in dairy products.

Acknowledgements

This work was supported by the research grants from the Higher Education Research Promotion and National Research University Project of Thailand, Office of the Higher Education Commission, Ministry of Education, Thailand.

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.

Altschul, Altschul, S. F., Madden, T. L., Schaffer, A. A., Zhang, J., Zhang, Z., Miller, W. and Lipman, D. J. 1997. Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. Nucleic Acids Research 25: 3389-3402.

Brosius, J., Ullrich, A., Raker, M. A., Gray, A., Dull, T. J., Gutell, R. R. and Noller, H. F. 1981. Construction and fine mapping of recombinant plasmids containing the rrnB ribosomal RLNA operon of *E. coli.* Plasmid 6: 112-118.

Buddington, R. 2009. Using probiotics and prebiotics to manage the gastrointestinal tract ecosystem. In Charalampopoulos, D. and Rastall, R. A. (Eds). Prebiotics and Probiotics Science and Technology, p. 1-31. New York: Springer Science.

Chen, C. S., Hsu, C. K. and Chiang, B. H. 2002. Optimization of the enzymic process for manufacturing low-lactose milk containing oligosaccharides. Process Biochemistry 38(5): 801–808.

Chockchaisawasdee, S., Athanasopoulos, V. I., Niranjan, K. and Rastall, R. A. 2005. Synthesis of galacto-oligosaccharide from lactose using beta-galactosidase from *Kluyveromyces lactis*: studies on batch and continuous UF membrane-fitted bioreactors. Biotechnology and Bioengineering 89(4): 434–443.

Chun, J., Lee, J. H., Jung, Y., Kim, M., Kim, S., Kim, B. K. and Lim, Y. W. 2007. EzTaxon: a web-based tool for the identification of prokaryotes based on 16S ribosomal RNA gene sequences. International Journal of Systematic and Evolutionary Microbiology 57: 2259-2261.

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.

Gosling, A., Stevens, G. W., Barber, A. R., Kentish, S. E. and Gras, S. L. 2010. Recent advances refining galactooligosaccharide production from lactose. Food Chemistry 121: 307-318.

Goulas, A. K, Kapasakalidis, P. G., Sinclair, H. R., Rastall, R. A. and Grandison, A. S. 2002. Purification of oligosaccharides by nanofiltration. Journal of Membrane Science 209(1): 321-335.

Hernandez, O., Muiz-Matute, A. I., Olano, A., Moreno, F. J. and Sanz, M. L. 2009. Comparison of fractionation techniques to obtain prebiotic galactooligo-saccharides. International Dairy Journal 19: 531-536.

Hsu, C. A., Yu, R. C. and Chou, C. C, 2005. Production of β-galactosidase by *Bifidobacteria* as influenced by various culture conditions. International Journal of Food Microbiology 104: 197-206.

Katsura, K., Kawasaki, H., Potacharoen, W., Saono, S., Seki, T., Yamada, Y., Uchimura, T. and Komagata, K. 2001. *Asaia siamensis* sp. nov., an acetic acid bacterium in the α-proteobacteria. International Journal of Systematic and Evolutionary Microbiology 51: 559-563.

Kawasaki, H., Hoshino, Y., Hirata, A. and Yamasato, K. 1993. Is intracytoplasmic membrane structure a generic criterion? It does not coincide with phylogenetic interrelationships among photosynthetic purple non-sulfur bacteria. Archives of Microbiology 160: 358-362.

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.

Sangwan,V., Tomar, S. K., Singh, R. R. B., Singh, A. K. and Ali, B. 2011. Galactooligosaccharides: novel components of designer foods. Journal of Food Science 76(4): 103-111.

Splechtna, B., Nguyen, T. H., Steinbock, M., Kulbe, K. D., Lorenz, W. and Haltrich, D. 2006. Production of prebiotic galacto-oligosaccharides from lactose using beta-galactosidases from *Lactobacillus reuteri.* Journal of Agricultural and Food Chemistry 54(14): 4999–5006.

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 and Sons Press.

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.

Yamada, Y., Katsura, K., Kawasaki, H., Widyastuti, Y., Saono, S., Seki, T., Uchimura, T. and Komagata, K. 2000. *Asaia bogorensis* gen. nov., sp. nov., an unusual acetic acid bacterium in the α-proteobacteria. International Journal of Systematic and Evolutionary Microbiology 50: 823-829.

Zhang, P., Timakov, B., Stankiewicz, R. L., Turgut, I. Y. 2000. A trans-activator on the *Drosophila* Y chromosome regulates gene expression in the male germ line. Genetica 109(1-2): 141-150.