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Evaluation of transgalactosylation activity of commercial β-galactosidase from *Bifidobacterium bifidum* for synthesis of prebiotic oligosaccharides

Milano, A. M. T. and *Arreola, S. L. B.

Institute of Chemistry, University of the Philippines Los Baños, College Laguna, 4031 Philippines

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Abstract

Galacto-oligosaccharides (GOS) are products of transgalactosylation reaction of β-galactosidase when lactose is used as the substrate. These carbohydrates are considered prebiotics which stimulate beneficial effects to human health. In the present work, Saphera, a commercial preparation of β-galactosidase from *Bifidobacterium bifidum*, was biochemically characterised for production of GOS. Using *o*-nitrophenyl-β-D-galactopyranoside (*o*NPG) as the substrate, optimal activity for the enzyme was found to be at pH 6.0 and at 45°C. Ten (10) mM each of either Na^+ or K^+ enhanced enzyme activity by 10%, while Cu^{2+}, Zn^{2+}, Fe^{2+}, and EDTA showed inhibitory effect on the enzyme activity. When incubated in 50 mM sodium phosphate and pH 6.5, the enzyme was found to have half-life time of 136 ± 6 and 2.1 ± 0.2 h at 30 and 50°C, respectively. The hydrolysis activity of the enzyme predominated when the initial lactose concentration used was 5% (w/v). When initial lactose concentration was increased to 20% (w/v), maximum GOS yield obtained was 10% (w/w) achieved at 86% lactose conversion. Analysis using high performance anion exchange chromatography with pulsed amperometric detection (HPAEC-PAD) revealed that the major oligosaccharides produced by the enzyme were 3'-galactosylglucose, 3'-galactosyllactose, 3'-galactobiose, and allolactose, thus showing that this enzyme would prefer to form *β*-(1→3)- and *β*-(1→6)-linked GOS. Despite its predominantly hydrolytic activity, Saphera synthesised prebiotic GOS which could be interesting to dairy industry.

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Introduction

For years, food industry uses the enzyme β-galactosidase (EC 3.2.1.23, lactase) in various purposes. This enzyme hydrolyses the β-1,4-glycosidic bond of milk sugar (lactose) to produce glucose and galactose (Pivarnik *et al*., 1995). This reaction is commonly used in the dairy industry to produce lactose-free or low-content lactose products, and in cheese whey production to minimise environmental problems (Mlichova and Rosenberg, 2006; Oliveira *et al*., 2011; Kittibunchakul *et al*., 2020). Food manufacturers and scientific researchers have shown great interest on β-galactosidases due to the enzyme's propensity to synthesise galacto-oligosaccharides (GOS) that have beneficial health effects to human (Lamsal, 2012; Rodriguez-Colinas *et al*., 2014). These include protection against enteric infection, prevention of allergies, and better mineral absorption (Torres *et al*., 2010).

During the initial stage of catalysis of β-galactosidase where lactose is used as the substrate, a covalent galactosyl-enzyme intermediate is formed. The galactose from the complex is then transferred to a galactosyl acceptor which is present in the reaction mixture. Water could act as an acceptor, thus favouring lactose hydrolysis to form glucose and galactose. In addition, the β-galactosidase could possess transgalactosylation activity, where the galactosyl moiety from the galactosyl-enzyme intermediate is transferred to other carbohydrates (lactose, glucose, galactose, *etc*.) present in the

*Corresponding author.
Email: sbarreola@up.edu.ph

solution, thus forming GOS of different lengths or degree of polymerisation (DP) as reaction products (McCarter and Withers, 1994). To date, there are more than 40 different GOS that are structurally characterised (Gänzle, 2022). These known GOS have DP up to eight, which is commonly joined together *via* β−(1→3), β−(1→4), β−(1→6), and β−(1→2) glycosidic bonds (Torres *et al*., 2010; Kittibunchakul *et al*., 2020).

GOS fulfil the criteria of prebiotics (Sangwan *et al*., 2011; Crittenden, 2012; Rao *et al*., 2021). Prebiotic is defined as a substrate that is selectively utilised by host's microorganisms, thus conferring health benefit (Gibson *et al*., 2017). In the human digestive tract, the digestive enzymes slowly hydrolyse GOS which are then fermented by the colonic bacteria to produce short-chain fatty acids (SCFAs), CO_2, and H_2 (Gänzle, 2022). Acetic, butyric, and propionic acids are the most common SCFAs produced during carbohydrate fermentation. Isobutyrate, isovalerate, and 2-metyl butyrate obtained from the breakdown of some amino acids can also be found in the colon, albeit in lesser amount (Rios-Covian *et al*., 2016). High levels of SCFAs are concomitant to decrease in luminal pH which could result in the inhibition of growth of pathogenic microorganisms and high nutrient absorption (Macfarlane and Macfarlane, 2012).

There are various sources of β-galactosidases including plants and animals (Saqib *et al*., 2017). Recently, researchers focus on the use of microbial β-galactosidases particularly from bifidobacteria and lactobacilli since they are generally recognised as safe (GRAS) and easy to produce (Gosling *et al*., 2010; Saqib *et al*., 2017). Saphera, a commercial preparation of β-galactosidase from *Bifidobacterium bifidum*, is currently used in the production of low-lactose dairy products (Novozymes, 2022). Relative studies on this enzyme preparation showed that higher initial lactose concentrations (40 - 47%, w/v) were used in the formation of GOS (Guerrero *et al*., 2015; Füreder *et al*., 2020). Since transgalactosylation is affected by process conditions such as initial substrate concentration, our aim was to evaluate the propensity of this enzyme preparation to synthesise GOS even at lower initial lactose concentrations. In addition, we expected that the enzymatically synthesised GOS by *B. bifidum* β-galactosidase will be consumed preferentially by bifidobacteria and lactobacilli present in the human gut (Kittibunchakul *et al*., 2020).

Materials and methods

Materials

All reagents used were commercially available, and of the highest grade. The *B. bifidum* β-galactosidase preparation (Saphera 2600L) was provided by Novozymes (Copenhagen, Denmark). The substrate o-nitrophenyl-β-D-galactopyranoside (*o*NPG) and GOS standards were purchased from Carbosynth (Berkshire, UK). The standards used were as follows: 3'-galactosylglucose (*β*-D-Gal*p*-(1→3)-D-Glc), allolactose (*β*-D-Gal*p*-(1→6)-D-Glc), 3'-galactobiose (*β*-D-Gal*p*-(1→3)-D-Gal), 4'-galactobiose (*β*-D-Gal*p*-(1→4)-D-Gal), 6'-galactobiose (*β*-D-Gal*p*-(1→6)-D-Gal), 3'-galactosyllactose (*β*-D-Gal*p*-(1→3)-D-Lac), 4'-galactosyllactose (*β*-D-Gal*p*-(1→4)-D-Lac), and 6'-galactosyllactose (*β*-D-Gal*p*-(1→6)-D-Lac).

Standard assays

Enzyme activity was determined using a chromogenic substrate, *o*NPG (Nguyen *et al*., 2006). Briefly, a properly diluted enzyme solution (20 µL) was added to a 480 µL of 22 mM *o*NPG previously dissolved in 50 mM sodium phosphate buffer at pH 6.5. The reaction mixture was incubated at 30°C with agitation of 600 rpm using a digital thermal mixer (SCILOGEX, Connecticut, USA). After 10 min of incubation, the enzyme was deactivated by adding 750 µL of 0.40 M Na_2CO_3 to the reaction mixture. The absorbance of the resulting solution was measured at 420 nm using an UV-Vis spectrophotometer (UV Mini 1240, Shimadzu, Japan). A set of *o*-nitrophenol (*o*NP) standards (0 - 0.80 mM) was prepared in the same buffer, and their absorbances were measured at 420 nm. The amount of *o*NP released by the enzyme was measured based on the constructed calibration curve, while the β-galactosidase activity was determined using Eq. 1, where U = µmol of *o*NP released per minute. All enzyme activity measurements were performed in triplicate.

$$\text{β-galactosidase activity } \left(\frac{U_{oNP}}{mL}\right) = \frac{\text{oNP released, µmol}}{mL} \times \frac{1}{10\ \text{min}} \times \frac{500\ \mu L}{20\ \mu L} \times \text{Dilution factor} \quad \text{(Eq. 1)}$$

Protein concentration of the enzyme was evaluated following Bradford's method using bovine serum albumin (BSA) as standard (Bradford, 1976).

Kinetic parameters of the enzyme

Steady-state kinetic parameters such as v_{max} and K_m were obtained by evaluating the enzyme activity in different *o*NPG concentrations (0.25 to 25 mM). The mixture was incubated as previously described in the standard assay. Data were plotted into the Michaelis-Menten equation using SigmaPlot software (SPSS Inc., Chicago, IL, USA).

β-galactosidase activity at different pH's and temperatures

The effect of pH on enzyme activity was determined using the standard assay with 22 mM *o*NPG previously dissolved in Briton-Robinson buffer in the pH range of 3 - 10 (Nguyen *et al*., 2012).

The optimum temperature for the enzyme activity was evaluated from 25 - 70°C following the standard assay conditions. The enzyme's thermostability at different temperatures (30, 37, and 50°C) was also assessed following the standard assay condition. The percent residual activity was determined by getting the ratio of enzyme activity at certain time (i) to the activity of the enzyme at time 0 (o) as shown in Equation 2:

$$Residual\ activity\ (\%) = \frac{\beta-galactosidase\ activity_i}{\beta-galactosidase\ activity_o}\ x\ 100 \quad \text{(Eq. 2)}$$

Effect of cations and EDTA on enzyme activity

Monovalent (Na^+, K^+) and divalent (Cu^{2+}, Mg^{2+}, Zn^{2+}, and Fe^{2+}) cations were evaluated for their effect on the enzyme activity. Each cation was added to 22 mM *o*NPG previously dissolved in 50 mM bis-tris buffer (pH 6.5) under the standard assay condition. The final concentrations of each cation in the reaction mixture were 1 and 10 mM. The effect of chelating agent EDTA on the enzyme activity was also evaluated. The reaction mixture without the added cation or EDTA served as the control.

GOS formation

GOS synthesis was carried out in a 1.5 mL scale using two different initial lactose concentrations (5 and 20%, w/v) dissolved in 50 mM sodium phosphate buffer at pH 6.5. Next, 125 U_{oNP} of the enzyme was added in the reaction mixture, and the lactose conversion was conducted at 30°C with agitation of 300 rpm using a digital thermal mixer (SCILOGEX, Connecticut, USA). At certain time intervals, aliquots were taken to monitor the residual enzyme activity, and to determine the levels of carbohydrates in the solution using HPAEC-PAD. Crude GOS samples (20 μL) were diluted with 1980 μL of Milli-Q water, and injected on a Dionex DX-500 system workstation (Dionex, Amsterdam, The Netherlands). The chromatographic column used was CarboPac PA-1 analytical column (4 × 250 mm, 10 μm) which was connected to a CarboPac PA1 guard column. Chromatograms were processed using Chromeleon version 6.5 software (Dionex Corp., Sunnyvale, CA). Glucose galactose, allolactose, and lactose were separated using isocratic elution of 15 mM NaOH for 45 min with a flow rate of 1.0 mL/min. For other GOS separation, a gradient elution of Solution A (100 mM NaOH) and Solution B (100 mM NaOH with 150 mM NaOAc) was performed with 2% B from 0 to 10 min, 2% B to 48% B from 10 to 40 min, and then 48% B for 5 min followed by 100% B for 15 min. Before injecting the next sample, re-equilibration was performed with 98% A and 2% B for 15 min. Quantification was performed using GOS standards.

Statistical analysis

All measurements were conducted in triplicate. Results were presented as mean ± standard deviation.

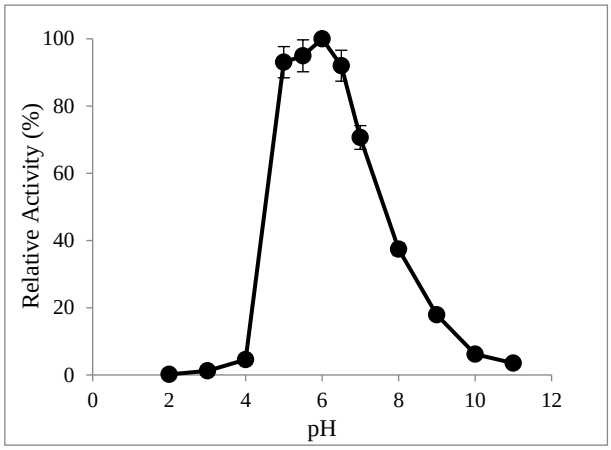
Results and discussion

Molecular characterisation of enzyme

In the present work, the propensity of commercial preparation, Saphera, was biochemically characterised towards the formation of GOS. The enzyme was found to have specific activity of 532 U_{oNP}/mg protein. Enzyme activity was found to be optimum at pH 6.0, and retained more than 90% of its initial activity when pH was between 5.0 and 6.5, and incubated for 10 min (Figure 1A). The optimum temperature was observed to be 45°C under standard assay conditions (Figure 1B). Our results were consistent with the study of Füreder *et al.* (2020) on Saphera where optimum pH range and temperature observed were 5.7 - 6.4 and 45°C, respectively.

Previously reported data for β-galactosidases from *B. bifidum* NCIMB41171 showed that the optimal pH was from 5.4 to 6.8 and optimal temperature was from 40 to 60°C when *o*NPG was used as a substrate (Goulas *et al*., 2009a). Commercial β-galactosidases preparation was shown to have different optimum pH's: β-galactosidases from *A. niger*, *A. aculeatus*, and *A. oryzae* were shown to have optimum pH from 3.5 to 4.5, while

those from *K. lactis* were found to have optimum at pH 7.0 (Guerrero *et al*., 2015). In liquid dairy products, β-galactosidases with optimum pH from 6 to 7 is desirable to efficiently hydrolyse lactose in milk products (Dekker and Daamen, 2011).



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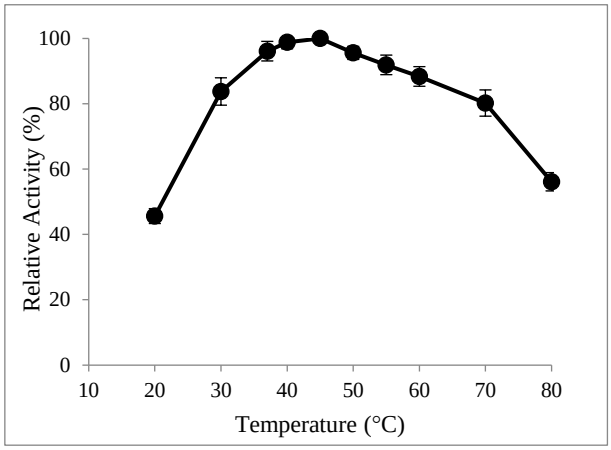


(B)

Figure 1. pH **(A)** and temperature **(B)** optimum of β-galactosidase from *B. bifidum* incubated in 20 mM Britton-Robinson buffer. Data are mean of three independent measurements (n = 3) with error bars indicating ± standard deviation.

Figure 2 shows the thermostability of *B. bifidum* β-galactosidase when incubated in 50 mM sodium phosphate buffer at pH 6.5. The enzyme retained more than 80% of its initial activity after 24 h of incubation, either at 30 or 37°C. However, about 20% of the initial activity was lost after 15 min of incubation, when incubated at 50°C in the same buffer. The half-life time of activity of the enzyme in 50 mM sodium phosphate buffer at pH 6.5 was found to be 136 ± 6, 80 ± 3, and 2.1 ± 0.2 h at 30, 37, and 50°C, respectively.

Cations have been shown to activate or inhibit enzyme activity. In the present work, Na^+ and K^+ were shown to stimulate enzyme activity when final concentration was 10 mM. However, final concentration of K^+ at 1 mM inhibited the enzyme activity by 10% (Table 1). This was consistent with the report of Goulas *et al*. (2009b) on β-galactosidase from *B. bifidum*. Previous report suggested that the monovalent cations Na^+ and K^+ affect enzyme activity by inducing conformational changes in the enzyme structure (Becker and Evans, 1969).

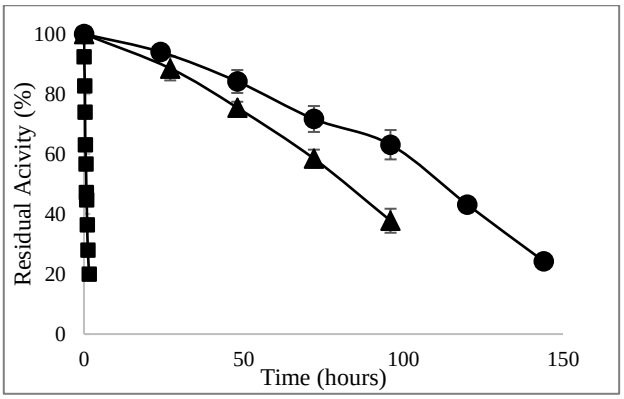


Figure 2. Stability of β-galactosidase from *B. bifidum* incubated in 50 mM sodium phosphate buffer with pH 6.5 at 30°C (●), 37°C (▲), and 50°C (■). Data are mean of three independent measurements (n = 3) with error bars indicating ± standard deviation.

Table 1. Effect of cations on β-galactosidase activity from *B. bifidum* in 10 mM Bis-tris buffer at pH 6.5.

Cation	Relative activity (%)	
	1 mM	10 mM
Blank (none)	100	100
Na^+	97 ± 1	110 ± 1
K^+	89 ± 1	111 ± 2
Mg^{2+}	104 ± 3	98 ± 2
Cu^{2+}	6 ± 1	< 1
Zn^{2+}	52 ± 1	< 1
Fe^{2+}	62 ± 1	< 1
EDTA	36 ± 1	36 ± 1

Values are mean ± standard deviation of three independent measurements (n = 3).

The addition of Mg^{2+}, on the other hand, did not affect the catalytic activity of the enzyme preparation. The addition of Cu^{2+}, Zn^{2+}, and Fe^{2+} caused an inhibitory effect on the enzyme activity especially upon increasing its concentration to 10

mM. Incubating the enzyme for 10 min in 50 mM sodium phosphate buffer with pH 6.5 at 30°C was shown to increase the enzyme activity by 50% when compared to that in bis-tris buffer of pH 6.5 under the same temperature. These showed the important role of Na^+ for enzyme catalysis or substrate binding (Whitaker, 2018). The addition of EDTA, either 1 or 10 mM, was shown to inhibit the activity of Saphera by 74%. EDTA was also shown to inhibit the activity of β-galactosidases from *B. infantis* and *Bacillus* sp. BY02 (Hung and Lee, 2002; Zhou *et al*., 2021).

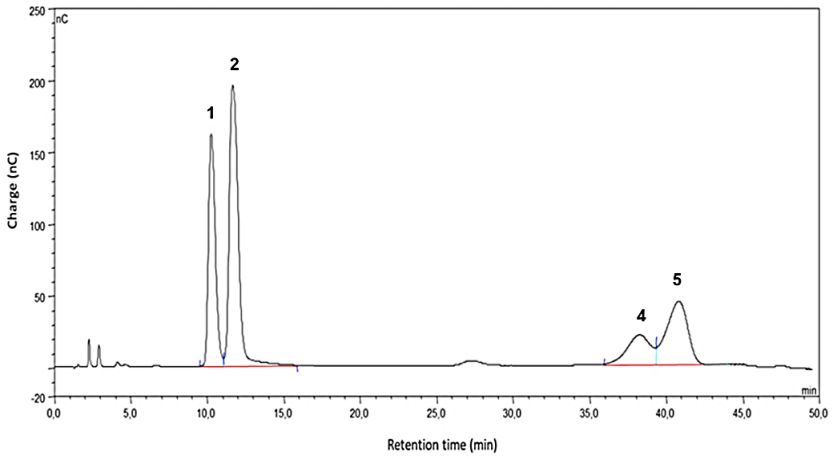
Using *o*NPG, the steady-state kinetic constants (K_m, and v_{max}) were determined. The Michaelis-Menten constant (K_m) was found to be 4.0 ± 0.2 mM, while maximum velocity (v_{max}) value was 532 ± 10 U/mg. v_{max} reported for this enzyme was higher when compared with β-gal from *B. longum* (70.67 U/mg) (Hsu *et al.*, 2005) and *B. breve* (486 U/mg) (Arreola *et al.*, 2014), but lower than β-gal from *B. bifidum* BbgI (1047 U/mg), BbgII (2721 U/mg), and BbgIV (590 U/mg) (Goulas *et al*., 2009a).

GOS synthesis

Based on the thermostability study, the β-galactosidase was stable at 30°C; hence, this temperature was used in the lactose conversion. The effect of initial lactose concentration and the incubation time on the GOS yield and product spectrum were first evaluated. Cow milk and other milk products such as low-fat, whole, and chocolate milk contain about 4 - 5% (w/w) lactose; thus, this lactose concentration was used (Johnson and Conforti, 2003). Using 5% (w/v) lactose dissolved in sodium phosphate at pH 6.5, 125 U_{oNP} of enzyme was added, and reaction was allowed to proceed for 2 h. The hydrolysis and transgalactosylation products during lactose conversion were analysed using HPAEC-PAD. After 30 min of reaction, 80% of the initial lactose concentration hydrolysed with glucose and galactose concentrations was about 23 g/L each. After 2 h of incubation, 1 g/L lactose remained unhydrolysed. Beside glucose and galactose, several transgalactosylation products were detected, albeit in minor concentration (less than 0.05 mg/L).

We increased the initial lactose concentration to 20% (w/v). This concentration was chosen due to its nearness to the solubility of lactose in water at 25°C, which is 220 g/L (Martins *et al*., 2019). Using 125 U_{oNP}, the hydrolysis and transgalactosylation activities of the enzyme were monitored by evaluating the carbohydrates present in the reaction mixture (Figures 3A and 3B). Throughout the 12-h lactose conversion, the enzyme retained at least 70% of its initial activity. At 48% lactose conversion (after 0.5 h of incubation), glucose and galactose were observed to be the major reaction products accounting for about 40% (w/w) of the total sugars (Figure 4). The 3'-galactosylglucose and 3'-galactosyllactose were found to be the predominant GOS; total concentration of which was 10 g/L. Maximum GOS yield of 20 g/L (or 10% of total sugar) was obtained when 86% of the initial lactose was hydrolysed. This yield corresponded to 10% w/w of the initial lactose concentration. At this point, 3'-galactosylglucose, 3'-galactosyllactose, 3'-galactobiose, and allolactose were observed to be the predominant transgalactosylation products, contributing approximately 74% of the total GOS produced (Figure 4). Other transgalactosylation products identified were 4'-galactosyllactose, 6'-galactosyllactose, and 6'-galactobiose. GOS concentration decreased thereafter since they were then used as hydrolysis substrate of the enzyme. At about 99% lactose conversion, the predominant allolactose (4 g/L) was found to be the predominant GOS. Other GOS were detected at this point, albeit in minor concentration.

Several factors could affect the yield and the product spectrum of β-galactosidases. These include enzyme used in the incubation, pH and temperature, lactose conversion, initial substrate concentration, and presence of activators or inhibitors of the enzyme (Splechtna *et al*., 2007; Rodriguez-Colinas *et al*., 2014; Martins *et al*., 2019). Several studies on Saphera showed different GOS yields under different reaction conditions. Füreder *et al*. (2020) found that 34.7% (w/v) initial lactose concentration yielded maximum GOS of 27%, achieved at 90% lactose conversion. In the same study, 3'-galactosyllactose (17.7 g/L) was found to be the most abundant GOS followed by allolactose (16.2 g/L), 3'-galactosylglucose (9.0 g/L), and 3'galactobiose (8.2 g/L). In the present work, concentrations at maximum GOS yield point of 3'-galactosylglucose and 3'-galactosyllactose were 7 and 5 g/L, respectively. In another study on Saphera, a maximum 15% (w/w) GOS was achieved when 40% (w/w) lactose in 0.05 M sodium buffer at pH 6.5 was used (Botvynko *et al*., 2019). Other commercial β-galactosidase preparations were shown to have maximum GOS



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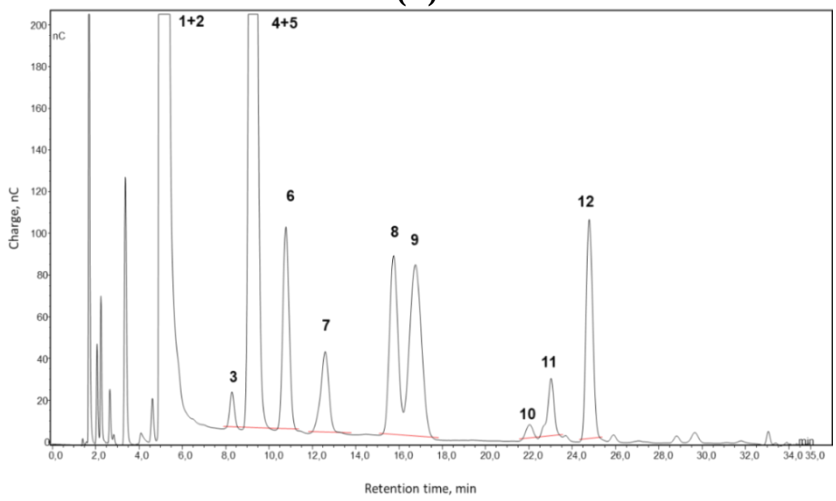


(B)

Figure 3. HPAEC-PAD analysis of glucose, galactose, allolactose, and lactose **(A)** and other GOS **(B)** synthesised by β-galactosidase from *B. bifidum*. Reaction condition: 125 U_{oNP} was incubated for 1.5 h in 20% (w/v) lactose dissolved in sodium phosphate buffer at pH 6.5. Identified compounds are (1) galactose, (2) glucose, (3) 6'-galactobiose, (4) allolactose, (5) lactose, (6) 6'-galactobiose, (7) 6'-galactosyllactose, (8) 3'-galactosylglucose, (11) 4'-galactosyllactose, and (12) 3'-galactosyllactose. Peaks 9 and 10 are unidentified.

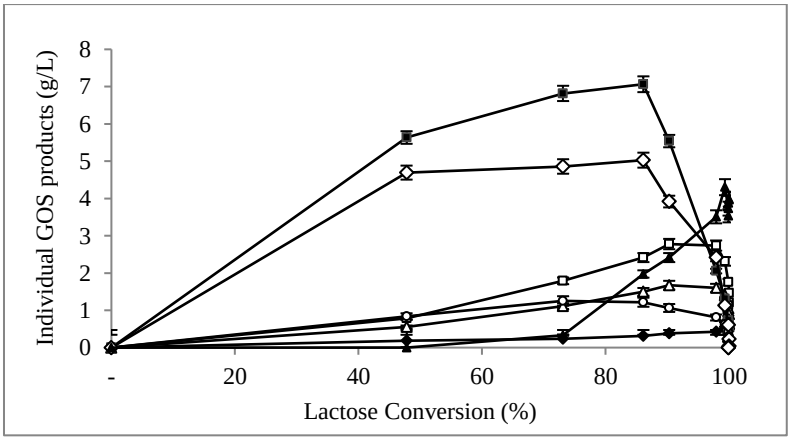


Figure 4. Concentration of individual galacto-oligosaccharides at different lactose conversions. Reaction condition: 125 U_{oNP} incubated in 20% (w/v) lactose dissolved in sodium phosphate buffer at pH 6.5. Legend: 3'-galactosylglucose (■), 3'-galactosyllactose (◊), 3'-galactobiose (o), allolactose (▲), 4'-galactosyllactose (●), 6'-galactosyllactose (Δ), and 6'-galactobiose (♦).

yields between 15 - 39% when 40% (w/w) lactose was used (Guerrero *et al*., 2015; Botvynko *et al*., 2019). Jørgensen *et al*. (2001) reported that β-galactosidase from *B. bifidum* synthesised 37- 44% GOS when initial lactose concentrations were between 10 - 40% (w/w). In the reports of Torres *et al*. (2010) and Fischer and Kleinschmidt (2018), higher initial lactose concentrations resulted in high GOS yield; however, yield saturation occurred when lactose concentration was greater than 30%.

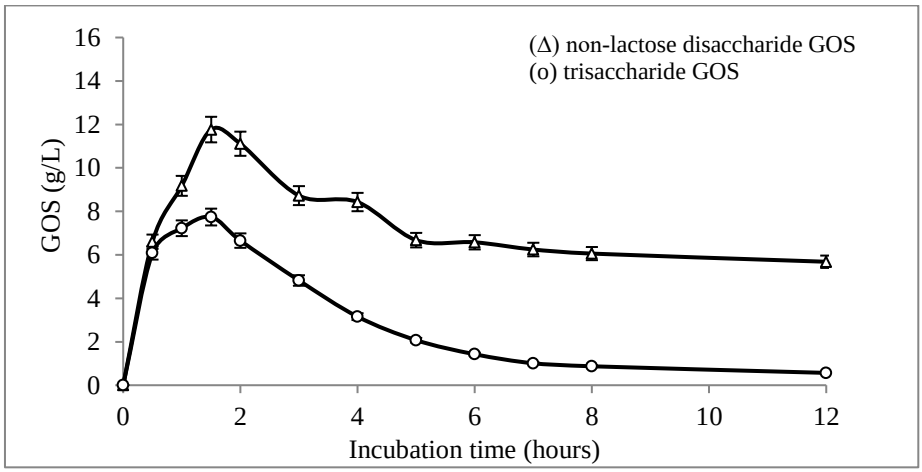
The extent of transgalactosylation of β-galactosidases is usually assessed by free glucose-to-galactose ratio. Value close to 1 indicates enzyme's preference to hydrolysis (Fischer and Kleinschmidt, 2018). In the present work, the ratio was found to be 1.00 - 1.02 during entire lactose conversion. However as shown in Figure 3, the enzyme was able to synthesise reasonable amount of GOS. Other methods used to evaluate transfer activity include the ratio of GOS at maximum yield point to the hydrolysed lactose (GOS/Lac) and maximum GOS to free galactose (GOS/Gal) (Fischer and Kleinschmidt, 2018). GOS/Lac ratio close to 1 indicates high transgalactosylation activity (Frenzel *et al*., 2015). In the present work, GOS/Gal and GOS/Lac were both found at 0.31. β-galactosidase from B. *bifidum* yielded 40% GOS with GOS/Gal and GOS/Lac of 2.7 and 0.5, respectively (Goulas *et al*., 2007; Fischer and Kleinschmidt, 2018). Other commercial β-galactosidases from *A. niger*, *K. lactis*, *A. oryzae*, *and B. circulans* were reported to have GOS yield of 11 - 25% with GOS/Lac values between 0.2 to 0.5 (Prenosil *et al*., 1987; Pocedičová *et al*., 2010; Fischer and Kleinschmidt, 2015; Rodriguez-Colinas *et al*., 2016).

The enzyme preparation Saphera was also shown to predominantly synthesise non-lactose disaccharides GOS (Figure 5A). At maximum GOS yield point, DP2 predominated with about 12 g/L (60% of total GOS). This was consistent with the study of Füreder *et al*. (2020) on Saphera where DP2 GOS was also found to be predominant. Report showed that probiotic bacteria in the human gut prefer to metabolise DP2 GOS (Böger *et al*., 2019a). The enzyme has been shown to have higher preference to synthesise β-(1→3)-linked GOS (Figure 4B). At maximum GOS yield point, about 75% (w/w) of the total GOS synthesised was found to be β-(1→3) linkages, while β-(1→6)- and β-(1→4)-linked GOS were about 19 and 6%, respectively. β-galactosidases from other bacterial sources showed different trends. β-galactosidase from *B. bifidum* (BgbII) preferentially synthesised β-(1→6)-linked GOS, while the recombinant β-galactosidase from *L. bulgaricus* and *B. breve* favour the formation of β-(1→6) and β-(1→3) linkages (Goulas *et al*., 2009b; Nguyen *et al*., 2012; Arreola *et al*., 2016).

Factors such as enzyme source, monomer composition, glycosidic linkage, and length influence prebiotic activity of GOS (Depeint *et al*., 2008; Logtenberg *et al*., 2020). In addition, the utilisation of these GOS is strain-specific (Thongaram *et al*., 2017; Böger *et al*., 2019b). In a study of Kittibunchakul *et al*. (2018), fermentability of GOS mixtures containing β(1→3) and β(1→6) linkages was found to be metabolised preferentially by eight *Lactobacillus* spp. and three *Bifidobacterium* spp. strains (Kittibunchakul *et al*., 2018). *In vitro* fermentation of GOS using piglet faecal inoculum showed preference for hydrolysis of β(1→2) and β(1→3) linkages over β(1→4) and β(1→6) linkages (Difilippo *et al*., 2016). *B. longum* was reported to partially utilise GOS of DP4 and DP5, while *B. adolescentis* and *B. infantis* were shown to have preferred GOS of DP3 and DP4, respectively (Barboza *et al*., 2009). Depeint *et al*. (2008) also reported that GOS mixture produced by the enzymes from *B. bifidum* exhibited higher bifidogenic and prebiotic effects than those GOS mixtures produced by *B. circulans* enzymes (Depeint *et al*., 2008).

Conclusion

The transgalactosylation reaction of the enzyme preparation Saphera was studied using two different initial lactose concentrations, 5 and 20% (w/v). When 5% initial lactose concentration was used, glucose and galactose were found to be the major products. Several GOS were detected, however they were in small amount. A higher maximum GOS yield of 10% (w/w) at 86% lactose conversion was observed when an initial lactose concentration of 20% (w/v) was used. At maximum GOS yield point, β(1→3)-linked GOS were most abundant as compared to β-(1→6) and β-(1→4) linkages, while non-lactose disaccharides predominated over trisaccharides. The present work concluded that Saphera was able to synthesise GOS even at 20% (w/v) initial lactose concentration. Therefore, it could be an interest to the food industry.



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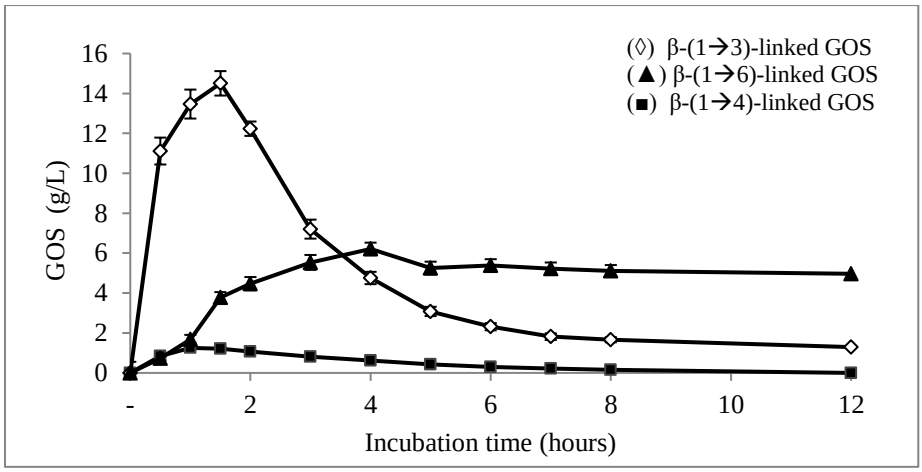

(B)

Figure 5. Formation and hydrolysis of GOS synthesised by β-galactosidase from *B. bifidum* based on degree of polymerisation **(A)** and on newly formed glycosidic linkages **(B)**. Reaction condition: 125 U_{oNP} incubated in 20% (w/v) lactose dissolved in sodium phosphate buffer at pH 6.5.

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