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Synthesis of β(1 → 3) and β(1 → 6) galactooligosaccharides from lactose and whey using a recombinant β-galactosidase from *Pantoea anthophila*

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ABSTRACT

Background: Milk whey, a byproduct of the dairy industry has a negative environmental impact, can be used as a raw material for added-value compounds such as galactooligosaccharides (GOS) synthesis by β-galactosidases.
Results: B-gal42 from *Pantoea anthophila* strain isolated from tejuino belonging to the glycosyl hydrolase family GH42, was overexpressed in *Escherichia coli* and used for GOS synthesis from lactose or milk whey. Crude cell-free enzyme extracts exhibited high stability; they were employed for GOS synthesis reactions. In reactions with 400 g/L lactose, the maximum GOS yield was 40% (w/w) measured by HPAEC-PAD, corresponding to 86% of conversion. This enzyme had a strong predilection to form GOS with β(1 → 6) and β (1 → 3) galactosyl linkages. Comparing GOS synthesis between milk whey and pure lactose, both of them at 300 g/L, these two substrates gave rise to a yield of 38% (60% of lactose conversion) with the same product profile determined by HPAEC-PAD.
Conclusions: B-gal42 can be used on whey (a cheap lactose source) to produce added value products such as galactooligosaccharides.
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1. Introduction

The galactooligosaccharides (GOS) are considered non-digestible carbohydrates (prebiotics), with health benefits supported by different scientific studies [1,2,3,4]. GOS are structurally related to oligosaccharides from human milk (HMOS), which are considered the best substitutes for newborns and infant formulations [5,6]. It was demonstrated that GOS added to these products regulate the intestinal microbiome and activate the immunological system as the same as human milk [7]. Recently, it has been found that GOS glycosidic structure and their prebiotic effect are correlated [8]. The relation between GOS structure and fermentative properties of these oligosaccharides has been studied, it was observed that probiotics prefer to metabolize β(1 → 6) and β (1 → 1) galactosyl residues, on the contrary, some lactobacilli and bifidobacteria did not metabolize β(1 → 4) GOS [9].

For industrial production of GOS, microbial β-galactosidases are commonly employed as they are robust biocatalysts with high production yield [10]. β-galactosidase (EC 3.2.1.23) has a wide distribution in nature and can be found in animals, plants, and microbial sources [11,12]. This enzyme catalyzes both the hydrolysis of β-galactosyl linkages and the synthesis of β-D-galactosides. The first catalytic step is the formation of a galactosyl-enzyme intermediate by a nucleophilic attack into the anomeric sugar carbon, which releases a glucose molecule. Then, a galactose moiety is transferred from the glycosyl-enzyme intermediate to a water acceptor molecule, releasing galactose (Lactose hydrolysis). However, if another carbohydrate serves as an acceptor, a transgalactosylation reaction occurs with the synthesis of galactooligosaccharides (GOS) or another galactoside [13,14,15]. The newly formed oligosaccharide can either be used by the enzyme as donor substrate or serve as an incoming acceptor, resulting in oligosaccharides containing a high polymerization

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degree (DP), it varies from 2 to 8 monosaccharide units, yielding different GOS mixtures.

The type of glycosidic linkages present in synthesized GOS varies in function of the β-galactosidase used. In general, a mixture of GOS composed of β(1 → 3), β(1 → 6), and β(1 → 4) linkages in different proportions is typically obtained [16,17,18,19]. The enzymatic yield of the GOS synthesis depends on the microbial enzyme, initial lactose concentration, and reaction conditions such as reaction time, pH, and temperature [20], and the highest GOS synthesis capacity is supposed to be kinetically determined between the rates of transglycosylation and hydrolysis [17,21]. Lactose rich substrates are typically used for industrial GOS production. In particular, whey (a byproduct of the cheese industry), the principal dairy industrial waste that has a negative environmental impact, can be used as a raw material for added-value compounds [22]. GOS production represents an attractive technology of whey valorization; most of the works have been conducted using supplemented or concentrated whey and several β-galactosidases from Lactobacillus, Aspergillus oryzae, Kluyveromyces lactis, Bacillus circulans and Streptococcus thermophilus [23,24,25,26].

Recently, different microorganisms were isolated and characterized for different biotechnological applications from "Tejuino," a traditional and typical corn fermented beverage in Mexico [27]. From these microorganisms, permeabilized cells of Pantoea anthophila strains (Bac 55.2 and Bac 69.1), which were used as biocatalysts for GOS synthesis with lactose as substrate, producing a β (1 → 3) and β(1 → 6) GOS mixture [28]. They displayed a high transgalactosylation activity comparable to some industrial enzymes, such as β-galactosidases from B. circulans. In this work, the responsible enzyme for the GOS synthesis of P. anthophila was found in silico in the P. anthophila genome (accession number KKB03129.1), cloning, and expression of the sequence in E. coli was carried out. This β-galactosidase belongs to the GH42 family, and the GOS synthesis profile was characterized by HPAEC-PAD, using two substrates; milk whey and pure lactose.

2. Materials and methods

2.1. Reagents

Chemicals were acquired from Sigma-Aldrich; all of them were of analytical grade. Sweet whey powder with 60–65% of lactose, was obtained from Kraft Foods. 6-O-β-galactosyl-galactose (6-galactobiose), 6-O-β-galactosyl-glucose (allolactose), 3-O-β-galactosyl-galactose (3-galactobiose), 4-O-β-galactosyl-galactose (4-galactobiose) and 4′-O-β-galactosyl-lactose were purchased from Carbosynth (Berkshire, UK). Some pure galactooligosaccharides were provided previously in other works [18,29,30].

2.2. Bacterial strains and plasmid vectors

The *P. anthophila* β-galactosidase gene (number of access **KKB03129.1**) synthesized by GenScript (New Jersey), the sequence was optimized by codon usage for *E. coli* cloning and expression into pET22b(+) vector to obtain the pET22b/Bgal42 construct. The expression vector pET22b/Bgal42 was converted to competent *E. coli* BL21 (D3) for enzyme production.

2.3. Recombinant β-galactosidase production

An *E. coli* BL21 (D3) overnight culture in LB was carried out, containing the expression plasmid pET22b/Bgal42 was inoculated into fresh TB medium with ampicillin (100 mg/mL). The cells were grown at 37°C and 200 rpm, and enzyme production was induced at an optical density at 600 nm (OD600) of 0.6–0.8 by adding isopropyl-β-D-thiogalactopyranoside (IPTG) in a final concentration of 1 mM and incubated further at 28°C. Cells were harvested after 48 h of post-induction growth, washed with a buffer of sodium phosphate (pH 7, 50 mM) and resuspended in 10% of the culture volume. The crude cell extract was obtained from disrupted cells by sonication were centrifuged (10,000 rpm, 20 min, 4°C) to remove the cell debris.

2.4. Purification of protein

Crude cell extract was charged onto a HisTRap HP column (5 mL), it was pre-equilibrated with a sodium phosphate buffer A (20 mM, pH 7.4) with 0.5 M NaCl and 5 mM imidazole. The elution of proteins was carried out (1 mL/min) by an imidazole linear gradient from 5 to 500 mM. Fractions with activity were pooled, then desalted and concentrated by Centrifugation filter tubes (3 kDa cutoff). The purified protein (β-galactosidase) was stored in a buffer of sodium phosphate (50 mM, pH 7) at 4°C.

2.5. β-Galactosidase assay

The β-galactosidase assay was carried out at 40°C with *o*-nitrophenyl-β-D-galactopyranoside (o-NPG) as substrate. 20 μL of β-galactosidase preparation with appropriate dilution was mixed with 480 μL of sodium phosphate buffer (50 mM, pH 7) with ONPG (22 mM). The reaction was stopped by adding Na2CO3 (750 μL at 0.4 M) during 10 min. *O*-nitrophenol (o-NP) released was detected by spectrophotometry (420 nm). The *o*-NP coefficient of molar extinction under these conditions was obtained (757 M^{-1} cm^{-1}). One μmol of o-NPG hydrolyzed per minute under the assay conditions was stated as one unit of enzymatic activity. All experiments were carried out in triplicate.

2.6. Enzyme stability to pH and temperature

The maximal activity due to temperature was determined by measuring the enzymatic activity within a temperature range of 20–60°C. To measure the thermal stability of crude recombinant Bgal42 β-galactosidase the reaction was carried out in a buffer of sodium phosphate (50 mM, pH 7.0) at 30, 40, and 50°C. Samples were taken at specific time intervals. The residual o-NPG activity was determined under the conditions described above. The optimal pH was determined using Britton–Robinson aqueous universal buffer solution pH = 2–12 [31]. At each pH, the enzyme activity was measured under standard assay. All the experiments were performed in triplicate.

2.7. Effect of ions on enzymatic activity

To investigate the metal ions influence on enzyme activity (1, 10, and 100 mM), the following ions, K^{+1}, Na^{+1}, Mg^{+2}, Mn^{+2}, Fe^{+2}, Co^{+2}, and Cu^{+2} were added to enzyme solution with *o*-NPG as a substrate. These activities were compared to the enzymatic activity without metal ions under standard assay conditions. All the experiments were performed in triplicate.

2.8. Galactooligosaccharides synthesis

GOS synthesis reactions were accomplished at 50°C with crude recombinant β-galactosidase Bgal42 from *P. anthophila*, using lactose (400 g/L) and 15 U (ONPG/mL) of enzyme activity in a buffer of sodium phosphate (50 mM, pH 7.0). A comparison of the GOS profile obtained by this recombinant enzyme and the previous permeabilized cells as biocatalysts was carried out [28]. The reactions of Bgal42 from *P. anthophila* were performed using lactose and whey of the same lactose concentration. Sweet whey powder was dis-

solved in a buffer of sodium phosphate (50 mM, pH 7.0) to reach lactose concentration corresponding to approximately 300 g/L (this was the highest soluble concentration for whey powder). These reactions were carried out in 125 mL Erlenmeyer flasks using a reaction volume of 20 mL at 150 rpm and 50°C. After certain time intervals, samples were withdrawn, heated to 95°C for 10 min to inactivate the enzyme, then HPAEC-PAD analysis was performed. All experiments were performed in triplicate; data were expressed as mean values.

2.9. Galactooligosaccharides analysis by HPAEC-PAD

Samples were diluted and filtered appropriately by syringe filtration (0.22 μm) before HPAEC-PAD analysis. The reaction mixture composition was analyzed with a Dionex system (ICS3000), which consisted of a pump of SP gradient using a gold electrode (Ag/AgCl as reference electrode) coupled to autosampler (model AS-HV). The samples analysis was performed with the same conditions as used previously [28]. Helium was used for degassing all eluents. A Dionex Carbo-Pack PA-1 column (anion-exchange 4 × 250 mm) connected to a Carbo-Pack PA-1 guard column was used at 30°C. Milli-Q water with NaOH at 50% w/v was used as eluent. NaOH (15 mM) was used as at initial mobile phase (1 mL/min) during 28 min was; then, a NaOH gradient from 15 mM to 200 mM was performed in 7 min and finally, NaOH at a constant concentration (200 mM) for 25 min. Data acquisition was processed by a Chromeleon software. For the carbohydrates identification and quantification, commercially available standards were used or purified products described in previous papers.

3. Results and discussion

3.1. Expression and production of recombinant β galactosidase from P. anthophila

To date, no information is available on the biochemical and molecular characterization of *Pantoea* β-galactosidase. The β-galactosidase yield activity of *P. anthophila* permeabilized cells (Bac 55.2 and Bac 69.1), were certainly low (8–10 U/mL) [28]. Thus, an *in silico* search from the *P. anthophila* genome was carried out; it was found a GH42 family gene encoding β-galactosidase from the (accession number **KKB03129.1**), the synthetic gene was cloned into pET22b(+) vector for heterologous overexpression in *E. coli* BL21(DE3). The open reading frame (ORF) of the GH42 β-gal gene has 2061 bp, encoding 686 amino acids, corresponding to a protein of 77.9 kDa (predicted molecular mass).

The homology analysis of the amino acid sequence by BLAST showed 75–82% similarities with bacterial β-galactosidases, including *Erwinia, Serratia*, *Yersinia*, and *Cronobacter*. According to the results of multiple alignments, the acid/base and nucleophile sites of recombinant *P. anthophila* B-gal42 were estimated to be Glu157 and Glu314, respectively.

The activity of β-galactosidase in *E. coli* BL21(DE3) cells without induction was not detected; therefore, the enzyme activity obtained can be attributed to the recombinant enzyme. The production experiments were performed in shake flasks, and the activity of β-galactosidase reached 30 U/mL at 48 h post-induction growth. The recombinant β-galactosidase produced in *E. coli* was a soluble, intracellular, and histidine tagged protein. The enzyme was partially purified and used for GOS synthesis on lactose and whey.

3.2. Effect of temperature and pH on enzyme activity and stability

Most bacterial β-galactosidases employed for GOS synthesis generally exhibit maximal activity at slightly acidic or neutral pH [21]. In this work, the optimum pH of the recombinant *P. anthophila* B-gal 42 activity was 7.0, which was similar to the optimum pH of a β-gal of *Paenibacillus barengeltzzi* (GH2) [32], and lower than alkaliphilic β-galactosidase [32]. The optimum temperature measured under standard conditions was 60°C, which was higher than other β-galactosidases reported [21,33,34], including one from the same family GH42 (*Geobacillus stearothermophilus*) with an optimum of 37°C [35]. The activity was maintained at least 30 h at 50°C (Fig. 1a). Thus, this temperature was used for GOS synthesis. The β-galactosidase activity was lost when the pH was below 4 and above 10. The *P. anthophila* B-gal42 was stable at a pH from 6 to 8 with oNPG as substrate (Fig. 1b).

3.3. Effect of ions on β-galactosidase activity

Certain ions affected enzyme activity; they can be inhibitors or activators, which influence GOS yield. The crude *P. anthophila* B-gal42 exhibited high activities, approximately 200% compared to control at 100 mM of Mg^{2+} and 10 mM of Mn^{2+}. K^+ and Na^+ cations showed a slightly positive effect, while a high inhibition of enzymatic activity was observed in the presence of Mn^{2+}, Ca^{2+}, Cu^{2+}, Co^{2+} and Fe^{2+}, all of them at 100 mM. Similar behavior is observed for the β-gal of *Pediococcus acidilactici*; the only difference was the action of Ca^{+2} as an activator [36]. The main cations in sweet whey are Na^+ (16.5 mM, K^+ (29.6 mM), Mg^{2+} (2.8 mM) and Ca^+ (8.4 mM) [37]; these cations increased the enzyme activity. Table 1 displays the relative enzyme activity determined at 1, 10, and 100 mM by mixing the appropriate salt to the reaction mixture compared with a blank sample with no added salt.

3.4. GOS production

In order to characterize with more detail, the GOS profile synthesized by transgalactosylation using recombinant *P. anthophila* B-gal42, pure lactose was used for synthesis, the composition of the reaction mixture was measured at different times using HPAEC-PAD. The chromatographic profile of reaction at 6 h (highest GOS concentration) is showed in Fig. 2, the presence of 13 components was identified, 10 of these as transgalactosylation products. Peaks 1, 2, and 5 were identified as galactose, glucose, and lactose, respectively. The galactooligosaccharides were identified using the standards; disaccharides such as allolactose [Gal-β(1 → 6)-Glc], 6-galactobiose [Gal-β(1 → 6)-Gal], 3-galactosyl-glucose [Gal-β(1 → 3)-Glc], and 3-galactobiose [Gal-β(1 → 3)-Gal], as well as the trisaccharides, 6′-galactosyl-lactose [Gal-β(1 → 6)-Gal-β(1 → 4)-Glc], 3′-galactosyl-lactose [Gal-β(1 → 3)-Gal-β(1 → 6)-Glc], 6-galactotriose [Gal-β(1 → 6)-Gal-β(1 → 6)-Gal] and 3′-galactosyl-allolactose [Gal-β(1 → 3)-Gal-β(1 → 6)-Glc]. The GOS profile from recombinant β-galactosidase Bgal42 is in agreement with the profile obtained using *P. anthophila* permeabilized cells [28]. Table 2 summarizes oligosaccharides composition (w/w) versus reaction time. At the highest GOS concentration (6 h), from the total of sugars, the mixed monosaccharides glucose and galactose corresponded to 46.7%, lactose to 13.3%, 6-galactobiose to 5.7%, allolactose to 20.2% and other GOS to 14% respectively. This enzyme belonging to the GH42 family has a synthesis preference for β(1 → 6) galactosyl linkages, then β(1 → 3) galactosyl linkages, which was also observed by β-galactosidases from *Aspergillus oryzae* and *Kluyveromyces lactis*, enzymes belonging to the GH35 family [38], different also than the profile produced by *Bifidobacteria* whole cells with principally β(1 → 3), followed by β(1 → 4) and β(1 → 6) galactosyl linkages [39]. At the beginning of the reaction, the disaccharide allolactose, and the trisaccharide 3′-galactosyl-lactose, were predominantly formed, consisting up to 77% of total GOS in this initial stage. At higher lactose conversion, there are more variety of galactosyl

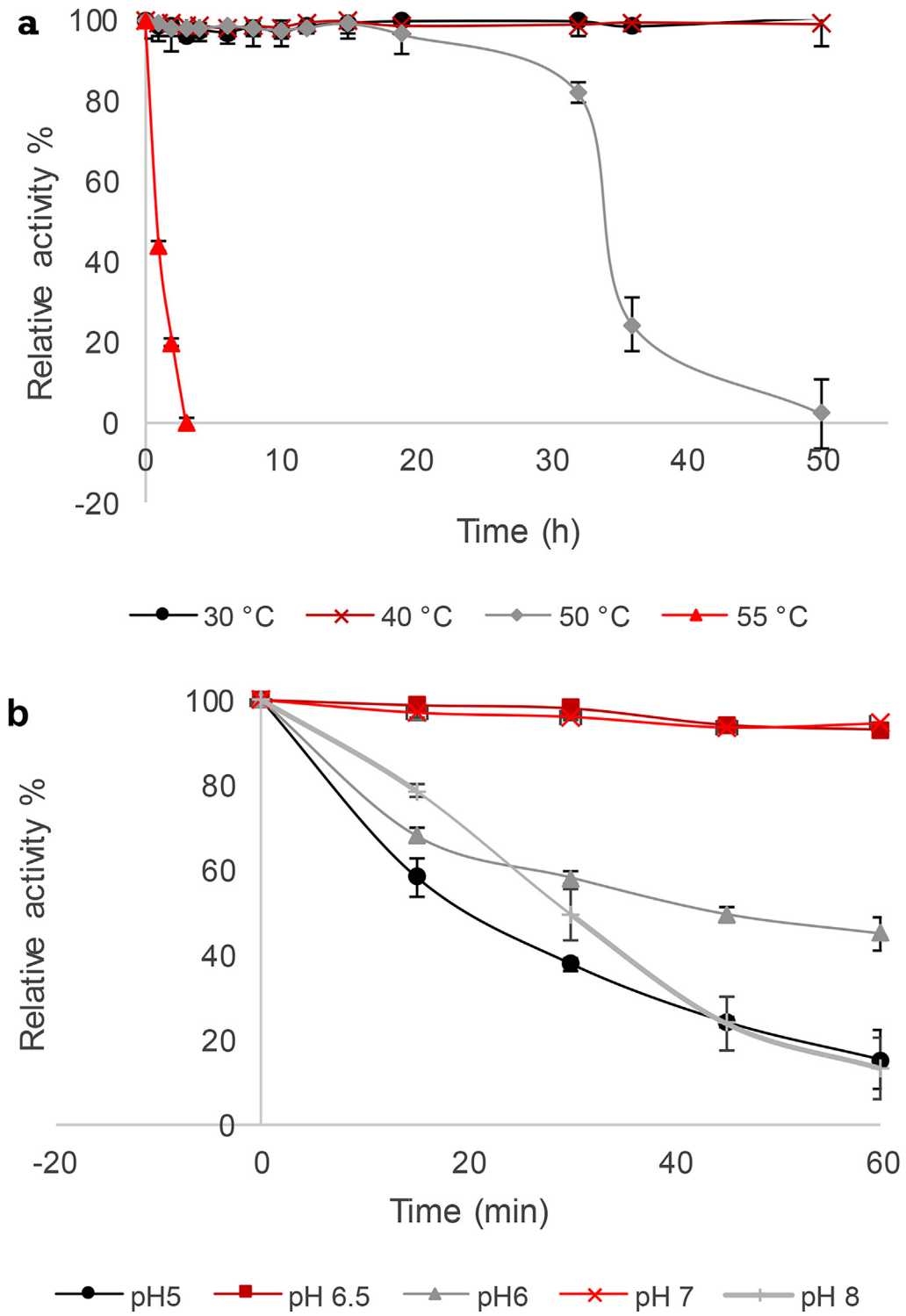

Fig. 1. Enzyme stability. (a) Temperature stability of the recombinant β galactosidase from *P. anthophila* determined at 30°C (●), 40°C (χ), 50°C (◆) and 55°C (▲) in 50 mM sodium phosphate buffer pH 6.5. (b) pH stability incubated 1 h in Britton-Robinson buffer pH 5 (●), pH 6 (■), pH 6.5 (▲), pH 7 (x) and pH 8 (+).

acceptors; therefore, GOS with different linkages were produced. The monosaccharides, glucose, and galactose increased concurrently with a reduction in lactose; however, the galactose was lower because it is incorporated into the different GOS structures. Approximately, at 6 h of reaction, the GOS yield reached the maximum value (159.9 g/L), corresponding to 40% of the total mixture of carbohydrates. Which was similar to the conversions and yields reported by β-galactosidases from the GH35 family [38,40,41] and lower than the values reported with a mutated GH35 β-galactosidase from *A. oryzae* [34] and a GH2 β-galactosidase from *Paenibacillu* [33].

According to Liu et al. [33], β-galactosidases from the GH2 family are better biocatalysts than β-galactosidases from the GH42 family. Comparing this recombinant β-galactosidase from *P. anthophila* with other reported bacterial β-galactosidases (Table 3), surprisingly, this GH42 family β-galactosidase showed a lactose

Table 1
Effect of cations on recombinant β-galactosidase from *P. anthophila*.[a]

Cation	Relative activity %		
	1 mM	10 mM	100 mM
Control	100	100	100
Na^+	120 ± 3.5	115 ± 3.5	107 ± 2.5
K^+	129 ± 3.7	119 ± 2.5	113 ± 2.2
Mg^{2+}	166 ± 1.4	172 ± 3.9	207 ± 3.5
Mn^{2+}	162 ± 2.6	200 ± 1.6	0 ± 0
Ca^{2+}	100 ± 3.7	141 ± 0.8	0 ± 0
NH_4^+	112 ± 1.6	128 ± 3.6	102 ± 3.8
Cu^{2+}	24 ± 3.3	0 ± 0	0 ± 0
Co^{2+}	143 ± 2.7	191 ± 1.1	0 ± 0
Fe^{2+}	167 ± 2.6	0 ± 0	0 ± 0

[a] Enzyme activity was determined under standard assay conditions using oNPG as substrate with the respective concentration of cation added; values are means ± standard deviation.

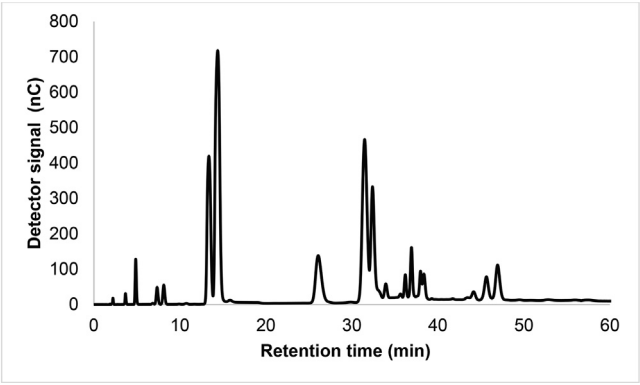

Fig. 2. HPAEC-PAD chromatogram of the reaction mixture at 6 h using β-galactosidase Bgal42 as biocatalyst for GOS synthesis. Peaks: (1) Galactose; (2) Glucose; (3) Gal-β(1 → 6)-Gal (6-galactobiose); (4) Gal-β(1 → 6)-Glc (allolactose); (5) Lactose; (6) Gal-β(1 → 3)-Gal (3-galactobiose); (7) Gal-β(1 → 6)-Gal-β(1 → 4)-Glc (6′-galactosyl-lactose); (8) Gal-β(1 → 6)-Gal-β(1 → 6)-Gal (6′-galactosyl-6-galactobiose); (9) Gal-β(1 → 3)-Glc (3-galactosyl-glucose); (10) Gal-β(1 → 4)-Gal-β(1 → 4)-Glc (4′-galactosyl-lactose); (11) Gal-β(1 → 3)-Gal-β(1 → 3)-Glc; (12) Gal-β(1 → 3)-Gal-β(1 → 6)-Glc (3′-galactosyl-allolactose); (13) Gal-β(1 → 3)-Gal-β(1 → 4)-Glc (3′-galactosyl-lactose).

conversion of 86% with GOS close to 40% in the carbohydrate mixture at the end of the reaction. It can be observed in Table 2 that GOS concentration decreased because hydrolysis prevailed over transgalactosylation; in this kinetically controlled reaction when the lactose is almost depleted, the oligosaccharides became hydrolyzed. This performance is exhibited by most β-galactosidases; GOS were accumulated as intermediates of lactose transgalactosylation and are hydrolyzed at a lactose conversion near to 100% [16]. According to literature, for most of the β-galactosidases, the maximum GOS yield is achieved at a lactose conversion between 50 and 85% [42,43,44]. The GOS yield achieved by the recombinant *P. anthophila* B-gal42 was similar to other bacterial β-galactosidases reported yields, from GH2 and GH35 families (30–40% w/w) [18,29,42,43,44,45,46].

Structures with β(1 → 6) linkage are difficult for the enzyme to hydrolyze; thus, GOS with this linkage are likely to accumulate. Allolactose is the major GOS structure during the entire reaction, and it decreased gradually after the point of maximum GOS yield. After 80% of lactose conversion, the disaccharides 6-galactobiose, allolactose, and 3-alactosyl galactose were the major transgalactosylation products in the mixture. Yin and collaborators [47] proposed that lactose isomers (allolactose and Glc(1 → 3) Gal) were synthesized by transgalactosylation from glucose as acceptor, rather than a glucose rearrangement from lactose in the active site.

It was reported that human consumption of β(1 → 3), β(1 → 4) and β(1 → 6) GOS produced by *B. bifidum* enzymes showed a better bifidogenic effect in comparison to commercial β(1 → 6) and β(1 → 4) GOS [4]. A recent study showed that 6-galactobiose and allolactose with β(1 → 6) linkages displayed similar *in vitro* bifidogenic properties in comparison to β(1 → 6) and β(1 → 4) GOS [8]. These studies attributed a bifidogenic effect for some of the GOS produced by β-gal42; hence this GOS mixture could be considered with substantial prebiotic potential.

3.5. GOS synthesis using sweet whey as lactose source

As initial lactose concentration is an important GOS yield factor [17]. The reaction was carried out using concentrated whey as a cheap lactose source. Whey powder is a commercial product of the dairy industry with a high lactose concentration. The whey powder was dissolved in a buffer of sodium phosphate (50 mM, pH 7), the maximum solubility was reached to an equivalent to 300 g/L of lactose concentration. In order to compare GOS synthesis on whey versus pure lactose (control), the same concentration was dissolved of lactose (300 g/L) in the same sodium phosphate buffer (50 mM, pH 7). Reactions were carried out at 50°C with 15 UONPG/mL. The lactose conversion was slower than previous GOS synthesis, with the highest GOS yield of 38% at 12 h in both lactose concentrations (Fig. 3). At the highest GOS yield, the lactose conversion was 60% for whey lactose and 78% for lactose solution, respectively. Thus, the mineral concentration of whey did not affect the GOS yield for the recombinant enzyme. When comparing the transgalactosylation products of using lactose solution or whey the GOS profile produced is almost the same. The main difference between the two substrates was the concentration of glucose, galactose, and lactose. The GOS/reacted lactose ratio and glucose/-galactose ratio were higher for the reaction using sweet whey; thus, transgalactosylation was favored, possibly by the cation composition [37]. In most of the works, the use of complex natural

Table 2
Carbohydrate composition (g/L) of the transgalactosylation reaction mixture using lactose as substrate and β-galactosidase Bgal42 from *P. anthophila*.[a]

Reaction time (h)	Galactose	Glucose	Lactose	Allolactose	6-Galactobiose	6′-Galactosyl lactose	6-galactotriose	3-Galactosyl galactose	3′-galactosyl allolactose	3′-Galactosyl lactose	Other GOS	TOTAL GOS
0	0	0	400	0	0	0	0	0	0	0	0.0	0.0
2	45.5	64.8	180.7	62.8	10.5	3.1	3.2	2.1	3.2	20.6	3.5	109.0
4	68.6	98.8	88.0	76.2	19.1	3.9	6.6	4.4	7.2	20.4	6.8	144.6
6	82.3	104.6	53.2	80.7	22.6	4.0	8.0	5.1	7.5	17.8	14.2	159.9
8	96.9	124.4	35.9	69.6	23.7	3.7	8.6	4.5	7.6	14.0	11.1	142.9
10	100.4	132.8	24.6	66.9	26.8	3.6	9.6	4.9	7.5	9.2	13.6	142.2
12	106.7	139.9	17.5	61.0	27.0	3.8	10.1	4.9	7.1	7.3	14.6	135.9
16	121.4	149.2	10.7	48.4	26.5	3.4	10.1	3.9	5.2	3.5	17.5	118.6
20	129.4	154.2	3.5	45.9	24.3	2.4	4.1	10.5	3.2	4.1	18.7	113.0

[a] Experimental conditions: 400 g/L lactose in 50 mM sodium phosphate buffer (pH 7), 15 U/mL, 50°C.

Table 3
Comparison of GOS synthesis with LAB and commercial β-galactosidases.

β-Galactosidase source	Glycoside Family	Maximum GOS yield (%)	Lactose conversion (%)	Type of Linkage	Reference
Lactobacillus reuteri	GH2	38	80	β (1–6) β (1–3)	[43]
Lactobacillus Sp	GH2	30	84	β (1–6) β (1–3)	[47]
Lactobacillus pentosus	GH2	31	78	β (1–6) β (1–3)	[42]
Lactobacillus plantarum	GH2	41	85	β (1–6) β (1–3)	[41]
Lactobacillus sakei	GH2	41	77	β (1–6) β (1–3)	[44]
Pantoea anthophila	GH42	40	86	β (1–6) β (1–3)	*
Bacillus circulans	GH2	48	88	β (1–4)	[46]
Kluyveromyces lactis	GH2	35	92	β (1–6) β (1–3)	[46]
Aspergillus oryzae	GH2	20	70	β (1–6) β (1–3) β (1–4)	[46]

* This study.

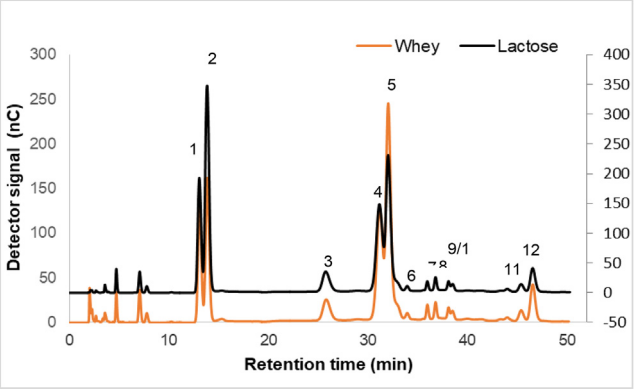

Fig. 3. HPAEC-PAD chromatograms of the reaction mixture at 12 h using sweet whey and lactose solution as substrates. Peaks: (1) Galactose; (2) Glucose; (3) Gal-β (1 → 6)-Gal (6-galactobiose); (4) Gal-β(1 → 6)-Glc (allolactose); (5) Lactose; (6) Gal-β(1 → 3)-Gal (3-galactobiose); (7) Gal-β(1 → 6)-Gal-β(1 → 4)-Glc (6′-galactosyl-lactose); (8) Gal-β(1 → 6)-Gal-β(1 → 6)-Gal (6′-galactosyl-6-galactobiose); (9) Gal-β (1 → 3)-Glc (3-galactosyl-glucose); (10) Gal-β(1 → 4)-Gal-β(1 → 4)-Glc (4′-galactosyl-lactose); (11) Gal-β(1 → 3)-Gal-β(1 → 3)-Glc; (12) Gal-β(1 → 3)-Gal-β (1 → 6)-Glc (3′-galactosyl-allolactose); (13) Gal-β(1 → 3)-Gal-β(1 → 4)-Glc (3′-galactosyl-lactose).

media reduced the fraction of higher GOS (DP4-DP6) [24], while only one study showed no influence in GOS yield and lactose conversion (and slight differences in GOS composition) using *L. lactis* β-galactosidase [48]. In a study with β galactosidase preparation Maxilact from *K. lactis*, the authors used recombined whey and lactose solution at an initial lactose concentration of 197 g/L; obtaining higher GOS yield with recombined whey (15.5%) compared with lactose solution (12.9%) [49]. Using Opti-lactase A50 from *A. oryzae* and an initial lactose concentration of 200 g/L, the GOS yield obtained was almost the same using lactose solution (24.3%), sweet whey (24.9%) and acid whey (24.5%) [37]. In this work, the whey components did not influence the GOS synthesis profile by the recombinant *P. anthophila* β-gal42; thus, sweet whey can be used to optimize GOS production at industrial level.

4. Conclusions

In this study, the transgalactosylation capacity of a new β-galactosidase from *Pantoea anthophila* was studied, this GH42 β-gal showed a high transglycosylation capacity, which has been normally reported for β-galactosidases from the GH2 family. *P. anthophila* B-gal42 showed similar behavior to those derived from LAB, having a noticeable preference for the synthesis of β (1 → 6) and β (1 → 3) linkages. The GOS profile produced by the *P. anthophila* B-gal42 was the same as the previous synthesis performed by the permeabilized cells of *P. anthophila*. Besides, we concluded that sweet whey is suitable for GOS synthesis with yields around 38% GOS, at an initial lactose concentration of 300 g/L displaying the same GOS profile that in lactose solution. The conversion of lactose whey into GOS could help to reduce dairy industry wastes by producing a high added value product, which can be used as a prebiotic food ingredient.

Conflict of interest

The authors declare no conflict of interest.

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Supplementary material

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