SELECTIVITIES OF THE β-GALACTOSIDASE OF *BACILLUS CIRCULANS* DURING THE PRODUCTION OF OLIGOSACCHARIDE PREBIOTICS

Clara L. de Oliveira Moreira[1], Luana Z. Weber[1], Nadia Krieger[2] & David A. Mitchell[1]*

[1] *Department of Biochemistry and Molecular Biology, Federal University of Paraná, Curitiba, Brazil.*
[2] *Department of Chemistry, Federal University of Paraná, Curitiba, Brazil.*
* *Corresponding author's email address: davidmitchell@ufpr.br*

ABSTRACT

There has been recent interest in using the β-galactosidase of *Bacillus circulans* to catalyze the production of two prebiotics, galactooligosaccharides (GOS) and lactulose, from a mixture of lactose and fructose. The selectivities of the enzyme for the various transgalactosylation and hydrolysis reactions that occur in this system determine the maximum yields of GOS and lactulose that can be produced, but reliable estimates of these selectivities are not available. In the current work, we demonstrate the use of a model-based method for determining these selectivities. The analysis shows that the β-galactosidase of *Bacillus circulans* has a 53-fold selectivity for producing GOS over lactulose and that GOS acts as a galactose donor for lactulose production late in the reaction. Our results also suggest that the secondary hydrolysis of GOS is significant. The selectivities estimated using our method are important parameters in time-based models, which can be used to guide the scale-up and optimization of enzyme bioreactors for the production of GOS and lactulose.

Keywords: β-galactosidase. Transgalactosylation. Lactulose. Galactooligosaccharides. Selectivity.

1 INTRODUCTION

The β-galactosidase of *Bacillus circulans* can be used to catalyze the production of two prebiotics, galactooligosaccharides (GOS) and lactulose (4-*O*-β-D-galactopyranosyl-D-fructofuranose), through the transgalactosylation of mixtures of lactose and fructose.[1] These prebiotics favor the growth of beneficial bacteria in the intestine, helping to prevent constipation, inhibiting the growth of pathogenic bacteria and reducing the risk of inflammatory bowel disease.[2,3]

If the galactooligosaccharides that are produced are treated as GOS3 (namely "Gal-Gal-Glc"), then six reactions can potentially occur when the β-galactosidase of *B. circulans* is added to a mixture of lactose and fructose:

Production of GOS3	$\mathrm{L + L \rightarrow G + T}$	(1)
Production of lactulose with lactose as the galactose donor	$\mathrm{L + F \rightarrow G + U}$	(2)
Production of lactulose with GOS3 as the galactose donor	$\mathrm{T + F \rightarrow L + U}$	(3)
Primary hydrolysis of lactose	$\mathrm{L + W \rightarrow G + g}$	(4)
Secondary hydrolysis of GOS3	$\mathrm{T + W \rightarrow L + g}$	(5)
Secondary hydrolysis of lactulose	$\mathrm{U + W \rightarrow F + g}$	(6)

In these reactions, L represents lactose, F represents fructose, G represents glucose, g represents galactose, T represents GOS3, U represents lactulose and W represents water. The yields of GOS and lactulose that are obtained depend on the selectivities of the enzyme for these different reactions. Several selectivities are important, not only the selectivity for the production of GOS in relation to lactulose, but also the selectivities for the production of the transgalactosylation products over the hydrolysis reactions. These selectivities are, therefore, important parameters for time-based mathematical models of the process, which will be important tools for guiding the scale-up and optimization of enzyme bioreactors for the production of these prebiotics.

Reliable estimates are not currently available for the selectivities of the β-galactosidase of *B. circulans* for the above transgalactosylation and hydrolysis reactions. The aim of the current work is to apply the model-based method for estimating enzyme selectivities, previously developed by Mitchell and Krieger,[4] to the production of GOS3 and lactulose in reactions catalyzed by the β-galactosidase of *B. circulans.*

2 MODEL

The model is written in terms of molar percentages of the species involved. The molar percentage for a generic species X is written as *X* (i.e. with the letter representing the species in italic font) and is defined as

$$X = 100 \times n_X / n_{Lo} \tag{7}$$

where n_X is the number of moles of species X and n_{Lo} is the initial number of moles of lactose.

The independent variable of the model is the percentage consumption of lactose, which is written as c and is given by

$$c = 100 - L \tag{8}$$

The parameters of the model are the selectivities of the enzyme, which are expressed relative to the reaction given in Eq. (2), namely the transgalactosylation reaction between lactose and fructose that produces lactulose. Five selectivities are defined: (i) R_{LL}, the selectivity for the production of GOS3; (ii) R_{TF}, the selectivity for the production of lactulose with GOS3 as the galactose donor; (iii) R_{LW}, the selectivity for the primary hydrolysis of lactose; (iv) R_{TW}, the selectivity for the secondary hydrolysis of GOS3; and (v) R_{UW}, the selectivity for the secondary hydrolysis of lactulose.

The β-galactosidase of *B. circulans* catalyzes the reactions shown in Eqs. (1) to (6) by the Ping Pong bi bi mechanism. Figure 1 shows the reactions in a scheme that is suitable for deducing kinetic equations for the system.

Given the reactions identified in Eqs. (1) to (6), the definition of molar percentage given in Eq. (7) and the definition of percentage consumption of lactose given in Eq. (8), it is possible to deduce the following set of balance equations:

Balance on lactose $$dL/dc = -1 \tag{9}$$

Balance on fructose $$dF/dc = (R_{UW}.U.W - L.F - R_{TF}.T.F)/D \tag{10}$$

Balance on glucose $$dG/dc = (R_{LL}.L.L + L.F + R_{LW}.L.W)/D \tag{11}$$

Balance on galactose $$dg/dc = (R_{LW}.L.W + R_{TW}.T.W + R_{UW}.U.W)/D \tag{12}$$

Balance on GOS3 $$dT/dc = (R_{LL}.L.L - R_{TW}.T.W - R_{TF}.T.F)/D \tag{13}$$

Balance on lactulose $$dU/dc = (L.F - R_{UW}.U.W + R_{TF}.T.F)/D \tag{14}$$

Balance on water $$dW/dc = (-R_{LW}.L.W - R_{TW}.T.W - R_{UW}.U.W)/D \tag{15}$$

Denominator "D" $$D = 2R_{LL}.L.L + L.F + R_{LW}.L.W - R_{TW}.T.W - R_{TF}.T.F \tag{16}$$

A program was written in Scilab, with Eqs. (9) to (16) being solved through numerical integration using the function *ode*. The selectivities R_{LL}, R_{TF}, R_{LW}, R_{TW} and R_{UW} were used as fitting parameters, with the function *fminsearch* being used to adjust the values of these selectivities so as to minimize the sum of squares of residuals between the experimental and predicted values of the molar percentages of both GOS3 and lactulose.

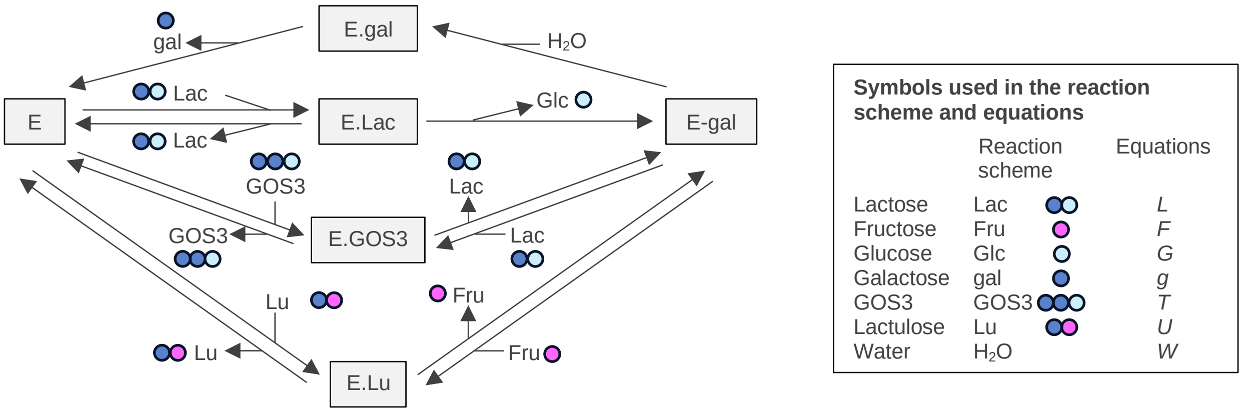


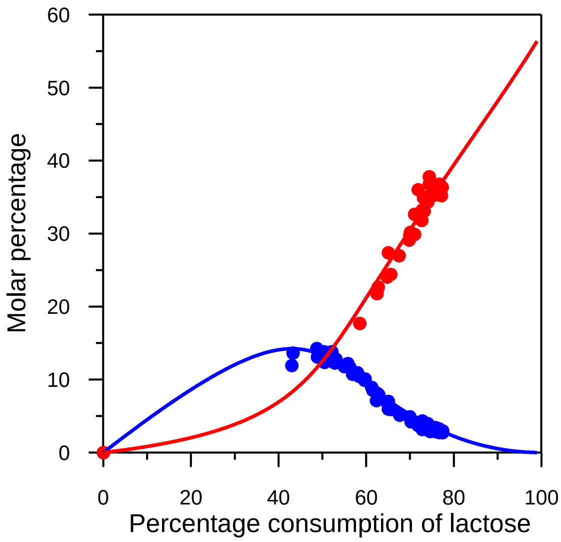
Figure 1 Representation of the reactions that occur during the production of lactulose and GOS3 from lactose and fructose, in a scheme highlighting the Ping Pong bi bi mechanism of the β-galactosidase of *Bacillus circulans*. Each of the reactions shown in Eqs. (1) to (6) is represented by a closed loop that begins and ends with the free enzyme (E) on the left hand side of the scheme.

3 RESULTS & DISCUSSION

The model was fitted to the data of Guerrero et al.[1] for the production of GOS3 and lactulose from a mixture of lactose and fructose, catalyzed by the β-galactosidase of *B. circulans*. Guerrero et al.[1] did not obtain data below 40% consumption of lactose, so it is not possible to check the fit of the model at the beginning of the reaction. Figure 2 shows that the model fits the data well above 40% consumption of lactose, with the selectivities given in the box of the figure. The value of R_{LL} indicates that the enzyme has a 53-fold selectivity for producing GOS3 over lactulose. However, since the initial molar ratio of fructose to lactose is 8:1, the initial rate of GOS3 production is only 6.6-fold greater than the initial rate of lactulose production. Lactulose production at the start of the reaction occurs solely via Eq. (2), Eq. (3) cannot occur at the start since there is no GOS3. The secondary hydrolysis of GOS3 is significant (R_{TW} = 1.2), but the very low values of R_{LW} (5.8×10^{-12}) and R_{UW} (4.1×10^{-13}) indicate that primary hydrolysis of lactose and secondary hydrolysis of lactulose are negligible. Importantly, the value of R_{TF} of 3.6 indicates that, late in the reaction, GOS3 is a significant donor of galactose for the production of lactulose, via Eq. (3).

Guerrero et al.[1] estimated the selectivity of the β-galactosidase of *B. circulans* for the production of the transgalactosylation products simply by dividing the number of moles of lactulose by the number of moles of GOS3. The estimated selectivities ranged from 0.9 at 43% lactose hydrolysis to 13 at 77% lactose hydrolysis. However, these values are not true selectivities, as they do not take into account the fact that the relative rates of different reactions depend not only on the selectivity of the enzyme itself, but also on the concentrations of the substrates of the reactions. In any case, the selectivities of the enzyme should remain constant during the reaction. Additionally, Guerrero et al.[1] did not explore the selectivities for the transgalactosylation reactions over primary and secondary hydrolysis.

Our model-based method for estimating the selectivities of the β-galactosidase of *B. circulans* is superior to the method of Guerrero et al.[1] Importantly, the selectivities estimated using our model remain constant during the reaction. Also, we identified that the secondary hydrolysis of GOS3 occurred. The selectivities estimated using our model can be incorporated into time-based models, which can be used to guide the scale-up and design of bioreactors for processes for the production of GOS and lactulose, using the β-galactosidase of *B. circulans.* Such models will also require the estimation of parameters related to the saturation, inhibition and denaturation of the enzyme. Mitchell and Krieger[4] presented a case study showing how this can be done.



All selectivities below are expressed relative to the reaction for the production of lactulose with lactose as the galactose donor (i.e. Eq. (2))

Reaction	**Estimated selectivity**
Production of GOS3	$R_{LL} = 52.8$
Production of lactulose with GOS3 as the galactose donor	$R_{TF} = 3.6$
Primary hydrolysis of lactose	$R_{LW} = 5.8\times10^{-12}$
Secondary hydrolysis of GOS3	$R_{TW} = 1.2$
Secondary hydrolysis of lactulose	$R_{UW} = 4.1\times10^{-13}$

The sum of squares of residuals was 91.1

Figure 2 Fit of the model to the data of Guerrero et al.[1] for the production of GOS and lactulose using immobilized β-galactosidase of *Bacillus circulans*. Guerrero et al.[1] used an initial molar ratio of fructose to lactose of 8:1 and a total initial sugar concentration of 50% (w/w). Key: (●) Experimental data for GOS and (●) Experimental data for lactulose. The box on the right shows the values of the selectivities that gave the best fit of the model to the data, with this fit being represented by the curves on the graph.

4 CONCLUSION

We have demonstrated the use of a model-based method for determining the selectivities of the β-galactosidase of *Bacillus circulans* during the production of galactooligosaccharides (GOS) and lactulose from a mixture of lactose and fructose. This enzyme has a 53-fold selectivity for producing GOS over lactulose, but significant lactulose concentrations are obtained because GOS acts as a galactose donor for lactulose production late in the reaction. Primary hydrolysis of lactose and secondary hydrolysis of lactulose were negligible, but secondary hydrolysis of GOS3 was not negligible. The estimated selectivities can be used in time-based models that can be used to guide the scale-up and optimization of enzyme bioreactors for the production of GOS and lactulose with the β-galactosidase of *B. circulans*.

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