SELECTIVITIES OF THE β-GALACTOSIDASE OF *ASPERGILLUS ORYZAE* DURING THE PRODUCTION OF OLIGOSACCHARIDE PREBIOTICS

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ABSTRACT

The β-galactosidase of *Aspergillus oryzae* can be used to catalyze the production of galactooligosaccharides (GOS) and lactulose, two prebiotic compounds, from a mixture of lactose and fructose. The maximum yields of GOS and lactulose that can be produced depend on the selectivities of the enzyme for the various transgalactosylation and hydrolysis reactions that occur in the system, however, the selectivities that have been estimated to date are not reliable. In the current work, we estimate these selectivities using a model in which the independent variable is the percentage consumption of lactose. The analysis shows that the β-galactosidase of *A. oryzae* is selective for producing GOS over lactulose. Further, as the initial molar ratio of fructose to lactose increases, three selectivities increase, relative to the transgalactosylation reaction that produces lactulose: (i) the selectivity for producing GOS; (ii) the selectivity for primary hydrolysis of lactose; and (iii) the selectivity for the secondary hydrolysis of GOS.

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

1 INTRODUCTION

The β-galactosidase of *Aspergillus oryzae* can be used to catalyze the production of two prebiotics, galactooligosaccharides (GOS) and lactulose (4-*O*-β-D-galactopyranosyl-D-fructofuranose), from mixtures of lactose and fructose.[1] If the GOS molecules that are produced are treated as GOS3 (namely "Gal-Gal-Glc"), then five reactions can occur in the system:

Production of GOS3 $$\mathrm{L + L \rightarrow G + T} \quad (1)$$

Production of lactulose $$\mathrm{L + F \rightarrow G + U} \quad (2)$$

Primary hydrolysis of lactose $$\mathrm{L + W \rightarrow G + g} \quad (3)$$

Secondary hydrolysis of GOS3 $$\mathrm{T + W \rightarrow L + g} \quad (4)$$

Secondary hydrolysis of lactulose $$\mathrm{U + W \rightarrow F + g} \quad (5)$$

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 selectivities of the enzyme for the reactions shown in Eqs. (1) to (5) determine the yields of GOS and lactulose that are obtained. Guerrero et al.[1] provided an estimate for the selectivity of the β-galactosidase of *A. oryzae* for the production of lactulose relative to the production of GOS, however, their estimate is not reliable. The aim of the current work is to obtain reliable estimates of the selectivities of the β-galactosidase of *A. oryzae*, not only for the production of lactulose relative to GOS, but also for the production of these transglycosylation products relative to primary and secondary hydrolysis. For this purpose, we used the model-based method for estimating enzyme selectivities that was previously developed by Mitchell and Krieger.[2]

2 MATERIAL & METHODS

The model is written in terms of the molar percentages of the species involved. For a generic species X, the molar percentage is written as *X* (i.e. in italic font) and is defined as:

$$X = 100 \times n_X / n_{Lo} \quad (6)$$

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

$$c = 100 - L \quad (7)$$

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

The β-galactosidase of *A. oryzae* catalyzes the reactions shown in Eqs. (1) to (5) 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 (5), the definition of molar percentage given in Eq. (6) and the definition of percentage consumption of lactose given in Eq. (7), the following set of balance equations can be deduced:

Balance on lactose	$dL/dc = -1$	(8)
Balance on fructose	$dF/dc = (-L.F + R_{UW}.U.W)/d$	(9)
Balance on glucose	$dG/dc = (L.F + R_{LL}.L.L + R_{LW}.L.W)/D$	(10)
Balance on galactose	$dg/dc = (R_{LW}.L.W + R_{TW}.T.W + R_{UW}.U.W)/D$	(11)
Balance on GOS3	$dT/dc = (R_{LL}.L.L - R_{TW}.T.W)/D$	(12)
Balance on lactulose	$dU/dc = (L.F - R_{UW}.U.W)/D$	(13)
Balance on water	$dW/dc = (-R_{LW}.L.W - R_{TW}.T.W - R_{UW}.U.W)/D$	(14)
Denominator "D"	$D = L.F + 2R_{LL}.L.L + R_{LW}.L.W - R_{TW}.T.W$	(15)

This system of equations was integrated using the *ode* function of Scilab and the *fminsearch* function of Scilab was used to estimate the values of the selectivities by using them as fitting parameters to fit the model to the data of Guerrero et al.[1] In some cases, the initial molar percentage of lactulose was not equal to zero and was estimated by extrapolating the experimental data back to the ordinate. The objective function was the sum of squares of differences between the experimental results and the fitted model, for both GOS3 and lactulose.

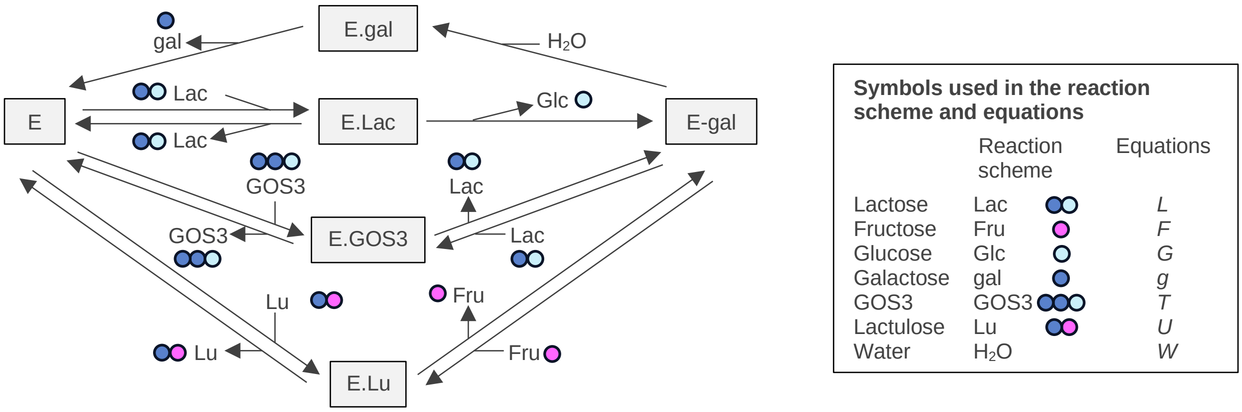

Figure 1 Representation of the reactions that occur during the production of lactulose and GOS3 from lactose and fructose, in a scheme that highlights the Ping Pong bi bi mechanism of the β-galactosidase of *Aspergillus oryzae*. Each of the reactions shown in Eqs. (1) to (5) is represented by a closed 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, catalyzed by the β-galactosidase of *A. oryzae*, using various initial fructose to lactose ratios. Good fits were obtained (Figure 2). The R_{LL} values are greater than 1 (Table 1), showing that the β-galactosidase of *A. oryzae* is selective for producing GOS in relation to lactulose. As the initial molar ratio of fructose to lactose increases, three selectivities increase: (i) The selectivity for GOS production (i.e. R_{LL}); (ii) the selectivity for primary hydrolysis of lactose (i.e. R_{LW}); and (iii) the selectivity for the secondary hydrolysis of lactose (i.e. R_{TW}). The selectivity for the secondary hydrolysis of lactulose (i.e. R_{UW}) is not affected by the initial molar ratio of fructose to lactose.

Guerrero et al.[1] estimated the selectivity for the production of lactulose in relation to GOS by dividing the number of moles of lactulose by the number of moles of GOS. However, selectivities estimated in this manner vary significantly during the reaction[3]. This occurs because the calculation does not account for the fact that the relative rates of the reactions producing lactulose and GOS depend not only on the selectivity of the enzyme itself, but also on the concentrations of fructose and lactose. The selectivities estimated in the current work are true selectivities: For a particular set of process conditions, the selectivity for each reaction in the system is characterized by a single numerical value. An additional limitation of the work of Guerrero et al.[1] is that they did not estimate the selectivities for the transgalactosylation reactions in relation to the primary and secondary hydrolysis reactions. Our model-based method allows estimation of these selectivities.

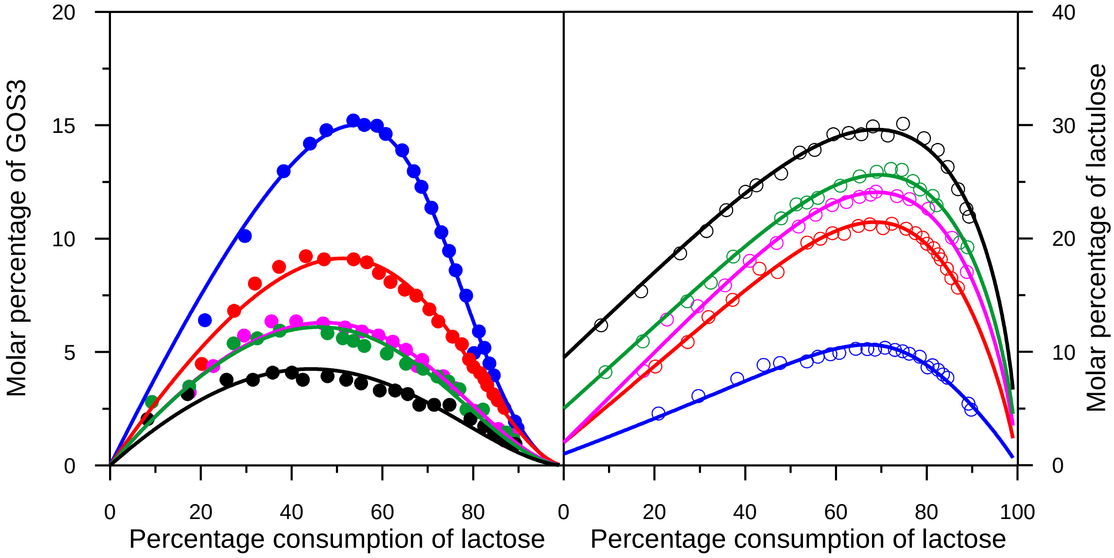

Figure 2 Fit of the model to the data of Guerrero et al.[1] for the production of GOS and lactulose using the β-galactosidase of *Aspergillus oryzae.* Guerrero et al.[1] used a total initial sugar concentration of 50% (w/w) and initial molar ratios of fructose to lactose of (●○) 1:1; (●○) 4:1; (●○) 6:1; (●○) 8:1 and (●○) 12:1. Key: Molar percentages of (solid symbols) GOS3 and (hollow symbols) lactulose. The curves represent the best fits of the model, with the values of the selectivities given in Table 1.

Table 1 Selectivities of the β-galactosidase of *Aspergillus oryzae*, relative to the reaction that produces lactulose, extracted from the data of Guerrero et al.[1]

Initial molar ratio of Fructose to Lactose (color used in Figure 2)	Selectivity for the reaction identified below, expressed relative to the reaction that produces lactulose			
	Production of GOS3 (R_{LL})	Primary hydrolysis of lactose (R_{LW})	Secondary hydrolysis of lactose (R_{TW})	Secondary hydrolysis of lactulose (R_{UW})
1:1 (blue)	2.6	0.010	0.12	0.098
4:1 (red)	3.4	0.016	0.15	0.095
6:1 (magenta)	3.4	0.024	0.19	0.095
8:1 (green)	4.9	0.033	0.24	0.094
12:1 (black)	5.1	0.046	0.27	0.090

4 CONCLUSION

In the current work, we determined the selectivities of the β-galactosidase of *Aspergillus oryzae*, for both transgalactosylation and hydrolysis reactions, during the production of galactooligosaccharides and lactulose from a mixture of lactose and fructose. Our estimates are true selectivities, unlike the previous estimates of Guerrero et al.[1] Our analysis shows that the β-galactosidase of *A. oryzae* is more selective for producing galactooligosaccharides than lactulose. Also, as the initial molar ratio of fructose to lactose increases, three selectivities increase, expressed relative to the reaction for production of lactulose: (i) the selectivity for production of GOS3; (ii) the selectivity for primary hydrolysis of lactose and (iii) the selectivity for secondary hydrolysis of galactooligosaccharies. The selectivity for the secondary hydrolysis of lactulose is not affected by the initial molar ratio of fructose to lactose.

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