Journal of Biotechnology 209 (2015) 31–40

Contents lists available at ScienceDirect

Journal of Biotechnology

journal homepage: www.elsevier.com/locate/jbiotec

Simultaneous synthesis of mixtures of lactulose and galacto-oligosaccharides and their selective fermentation

Cecilia Guerrero, Carlos Vera, Fernando Acevedo, Andrés Illanes*

School of Biochemical Engineering, Pontificia Universidad Católica de Valparaíso, Valparaíso, Chile

ARTICLE INFO

Article history:
Received 4 March 2015
Received in revised form 29 May 2015
Accepted 8 June 2015
Available online 12 June 2015

Keywords:
Lactulose
Galacto-oligosaccharides
Probiotics
Prebiotics
β-galactosidase

ABSTRACT

Lactulose and galacto-oligosaccharides (GOS) are well recognized prebiotics derived from lactose. In the synthesis of lactulose with β-galactosidases GOS are also produced, but the ratio of lactulose and GOS in the product can be tuned at will, depending on the operation conditions, so to obtain an optimal product distribution in terms of prebiotic potential. The selectivity of fermentation of each carbohydrate alone as well as mixtures of both was determined using pH-controlled anaerobic batch cultures with faecal inoculum. Within the experimental range considered, lactulose/GOS molar ratio of 4 resulted in the highest selectivity for *Bifidobacterium* and *Lactobacillus/Enterococcus*, so this ratio was selected as the target for the synthesis of lactulose from fructose and lactose with *Aspergillus oryzae* β-galactosidase. Synthesis was optimized using response surface methodology, considering temperature, initial concentrations of acceptor sugars and fructose/lactose molar ratio as key variables, with the aim of maximizing lactulose yield at the optimal product distribution in terms of prebiotic potential (lactulose/GOS molar ratio of 4). Under optimal conditions (50 °C, 50%w/w total initial concentrations of sugars and fructose/lactose molar ratio of 6.44), lactulose yield of 0.26 g of lactulose produced per g of initial lactose was obtained at the optimal product distribution.

© 2015 Elsevier B.V. All rights reserved.

1. Introduction

Prebiotics have emerged in recent years as a new category of functional foods (Gibson, 2006; Lamsal, 2012). Prebiotics are mostly non-digestible oligosaccharides that are selectively fermented in the colon, increasing the number of *Bifidobacterium* and *Lactobacillus* in the intestinal microbiota. These microorganisms are generally considered to be health promoting, inhibiting the growth of pathogenic bacteria and stimulating immunity, possibly increasing resistance to infections (Gibson, 2006; Pan et al., 2009; Lamsal, 2012). Short chain fatty acids (SCFA) are products of the saccharolytic activity of the intestinal microbiota and exert various effects by supplying energy to the intestinal mucosa, lowering pH and stimulating calcium and water absorption (Blottiere et al., 1999; Pryde et al., 2002; Pan et al., 2009). Several non-digestible oligosaccharides (NDOs) have been considered as potential prebiotics, namely: fructo-oligosaccharides (FOS), galacto-oligosaccharides (GOS), xylo-oligosaccharides (XOS), isomalto-oligosaccharides (IMO), soybean-oligosaccharides (SOS), lactulose (Lu) and inulin (IN) (Rycroft et al., 2001; Sanz et al., 2005; Rodriguez-Colinas et al., 2013). However, only a few satisfy all criteria to be considered as such, GOS and Lu being among them (Rycroft et al., 2001; Sanz et al., 2005; Gibson, 2006).

The enzymatic synthesis of GOS and Lu with β-galactosidases has been well documented (Albayrak and Yang, 2002; Lee et al., 2004; Kim et al., 2006; Vera et al., 2012). β-Galactosidase is a commodity enzyme used in the food industry for the production of low-lactose milk and dairy products. In recent years the enzyme, although being a hydrolase, has been used as a catalyst for transgalactosylation reactions and the synthesis of GOS and Lu with β-galactosidases has been well documented (Albayrak and Yang, 2002; Lee et al., 2004; Kim et al., 2006; Vera et al., 2012). This is feasible as long as the hydrolytic potential of the enzyme is depressed, which can be done by proper engineering of reaction conditions. In transgalactosylation, one molecule of galactose is transferred from the non-reducing end of a β-galactoside to a hydroxyl-bearing receptor molecule other than water. In the case of the synthesis of GOS with β-galactosidases, lactose acts both as donor and acceptor of the transgalactosylated galactose (Albayrak and Yang, 2002; Vera et al., 2012). In general, β-galactosidases are rather non-specific with respect to galactose acceptor molecules, so other sugars may act as such (Lee et al., 2004; Kim et al., 2006; Guerrero et al., 2013). In the synthesis of Lu with β-galactosidases, lactose is the galactosyl donor and fructose is the acceptor; however, since lactose and

* Corresponding author. Fax: +56 32 2273803.
E-mail address: aillanes@ucv.cl (A. Illanes).

http://dx.doi.org/10.1016/j.jbiotec.2015.06.394
0168-1656/© 2015 Elsevier B.V. All rights reserved.

fructose are present in the reaction medium, both can be acceptors of transgalactosylated galactose, so inevitably a mixture of Lu and GOS is produced (Guerrero et al., 2011). If the task is to produce pure Lu, this is a drawback; however, both NDOs are high added value products whose prebiotic properties are well established (De Preter et al., 2006; Rastall, 2010) so their mixture is likely to preserve or even enhance the prebiotic effect. It has been demonstrated that by proper manipulation of reaction conditions (mostly by varying the ratio of substrates) it is possible to obtain a wide range of Lu/GOS ratios (Guerrero et al., 2011). The hypothesis is that one of such ratios is likely to be optimum in terms of prebiotic effect; this is based on previous studies with other oligosaccharide mixtures, such as GOS/FOS and FOS/IN, where increases in the concentration of *Bifidobacterium* and *Lactobacillus/Enterococcus* have been reported to be higher than with each NDO used alone, so the prebiotic effect was enhanced (Vos et al., 2006; Ghoddusi et al., 2007). Since GOS formation is inevitable during the enzymatic synthesis of lactulose, the effect of such mixtures on the most numerically predominant and functionally relevant populations of the intestinal microbiota was assessed to appreciate their prebiotic potential. After determining the best Lu/GOS ratio, their simultaneous synthesis from lactose and fructose was carried out with *Aspergillus oryzae* β-galactosidase, evaluating the effect of temperature, fructose/lactose ratio and total sugars concentration on the corresponding Lu and GOS yields and productivities, and on Lu/GOS selectivity. Then yield of both prebiotics was optimized at a determined Lu/GOS ratio using response surface methodology.

2. Materials and methods

2.1. Materials

Lactulose (4-*O*-β-D-galactopyranosyl-D-fructose) was provided by Sigma Chemical Co. (St Louis, MO, USA). The commercial GOS preparation (BiMuno) was further purified to 99% GOS at the Department of Food and Nutrition Sciences of the University of Reading, UK and commercial GOS preparation Cup-Oligo (70% GOS) was supplied by Kowa Europe GmbH (Düsseldorf, Germany). *o*-Nitrophenol (*o*-NP) and *o*-nitrophenyl-β-D-galactopyranoside (*o*-NPG) and GOS standards (4β-galactobiose and 3α-4β-3α galactotetraose) were supplied by Sigma (St Louis, MO, USA). D-(+)-galactose, D-fructose, α-lactose monohydrate and all other reagents used for fermentation and enzymatic synthesis were analytical grade and provided either by Sigma, Merck (Darmstadt, Germany) or Oxoid Ltd (Basingstoke, UK).

A commercial β-galactosidase preparation of *Aspergillus oryzae*, under the trade name Enzeco® Fungal Lactase Concentrate, was a kind gift from Enzyme Development Corporation (New York, USA). Specific activity of the enzyme was 196,000 $IU_H\,g^{-1}$; one international unit of activity of β-galactosidase (IU_H) was defined as the amount of enzyme hydrolyzing 1 μmole of *o*-NPG per minute at pH 4.5, 40 °C and 30 mmol L^{-1} *o*-NPG. The enzyme was stored at 4 °C and retained full activity throughout the work period.

2.2. HPLC analysis of the reaction products

Substrates and products of synthesis were analyzed in a Jasco RI 2031HPLC system, provided with refractive index detector, isocratic pump (Jasco PU2080) and autosampler (Jasco AS 2055), using BP-100Ca^{++} columns (300 mm × 7.8 mm) for carbohydrate analysis (Benson Polymeric, Reno, USA). Samples were eluted with milli-Q water at a flow-rate of 0.5 mL min^{-1}. Temperatures in the column and in the detector were 80 °C and 40 °C, respectively. Chromatograms were integrated using the Jasco ChromPass software 1.7.403.1 (Maryland, USA). To determine sample composition, it was assumed that the area of each peak is proportional to the weight percentage of the respective sugar. This assumption was validated by a material balance. Standards of galactose, fructose, lactulose, 4β-galactobiose and 3α-4β-3α galactotetraose were used to determine their retention times and check linear range of the measurements.

2.3. Determination of prebiotic capacity in pure cultures

Bifidobacterium longum code NB667, *Bifidobacterium lactis* code Bb12 (ATCC 27536), *Lactobacillus plantarum* code CIDCA83114 and *Lactobacillus rhamnosus* code GC (ATCC53103). All strains were provided by the Research Centre in Food Cryobiology (CIDCA, La Plata, Argentina) being the selected strains for the determination of prebiotic capacity in pure cultures since these microorganisms are highly representative of the intestinal microbiota and considered as probiotics (Vernazza et al., 2006; Huebner et al., 2007; Vasiljevic and Shah, 2008). Fermentations were carried out at 37 °C, 280 rpm and pH 7 under anaerobic conditions in a series of 10 sealed vials to preserve anaerobic conditions, each vial corresponding to a sample point during fermentation. Culture medium was the one reported by Rycroft et al. (2001) and Vernazza et al. (2006). The basal medium contained in g L^{-1}: peptone water: 2; yeast extract: 2; NaCl: 0.1; K_2HPO_4: 0.04; KH_2PO_4: 0.04; $MgSO_4\,7H_2O$: 0.01; $CaCl_2\,6H_2O$: 0.01; $NaHCO_3$: 2; haemin: 0.05; L-cysteine: 0.5; HCl: 0.5; bile salts: 0.5; 2 mL L^{-1} of Tween 80 and 10 μL L^{-1} of vitamin K_1.

Due to the diversity of carbon sources, comparison among cultures was done on the basis of constant carbon atoms available for each substrate. Carbon sources evaluated were: lactulose, GOS (Cup Oligo) and mixtures thereof (at lactulose/GOS molar ratios of 0.5, 1 and 2). Glucose at 10 g L^{-1} was used as control carbon source, since it has no prebiotic effect and is rapidly metabolized by all the microorganisms tested. Cup Oligo contains 78.6 %w/w of GOS (Tri, tetra and pentasaccharides), 18.9% of disaccharides (GOS-2 and lactose) and less than 2% of monosaccharides.

2.4. pH-controlled batch cultures for determination of prebiotic capacity

Water-jacketed 80 mL fermenters were filled with 22.5 mL of the basal medium described above and inoculated with 2.5 mL of faecal slurry. All tested substrates, Lu, purified GOS (99%w/w) and mixtures of both (Lu/GOS molar ratio of 0.5, 1 and 4), were added separately just before inoculation to give a final concentration of 1% (w/v). Faecal slurries (10% v/v) were prepared by homogenizing freshly voided human faeces in 0.1 M phosphate buffer solution (PBS) pH 7. Each vessel was magnetically stirred and maintained under anaerobic conditions with oxygen-free nitrogen. Culture pH was controlled automatically during the fermentations at 6.8 and temperature was maintained at 37 °C (Rycroft et al., 2001; Sanz et al., 2005). Samples were removed from the fermenters at 0, 4, 8 and 24 h of incubation for enumeration of bacteria and determination of SCFA and lactic acid. The experiments were performed in triplicate, using one faecal sample from three different donors for each run of batch cultures with five different substrates each.

2.5. Enumeration of bacteria

Determination of biomass in pure cultures was done by turbidimetry at 650 nm and dry weight. In pH-controlled batch cultures fluorescent in situ hybridization technique (FISH) was used to quantify selected bacterial groups of the colonic microbiota. At 0, 4, 8 and 24 h of fermentation, 375 μL samples were removed from the fermenters and added to 1.125 mL of filtered 4% (w/v) paraformaldehyde solution pH 7.2, mixed and stored at 4 °C for 6 h to fix the cells. Hybridization of the samples was carried out

Download English Version:

https://daneshyari.com/en/article/6491035

Download Persian Version:

https://daneshyari.com/article/6491035

Daneshyari.com