International Dairy Journal 38 (2014) 11–15

Contents lists available at ScienceDirect

International Dairy Journal

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

Selective fermentation of potential prebiotic lactose-derived oligosaccharides by probiotic bacteria

Tomás García-Cayuela, Marina Díez-Municio, Miguel Herrero, M. Carmen Martínez-Cuesta, Carmen Peláez, Teresa Requena*, F. Javier Moreno

Instituto de Investigación en Ciencias de la Alimentación, CIAL (CSIC-UAM), CEI (UAM+CSIC), Nicolás Cabrera 9, 28049 Madrid, Spain

ARTICLE INFO

Article history:
Received 11 December 2013
Received in revised form
28 March 2014
Accepted 31 March 2014
Available online 2 May 2014

ABSTRACT

The growth of potential probiotic strains from the genera *Lactobacillus*, *Bifidobacterium* and *Streptococcus* was evaluated with the novel lactose-derived trisaccharides 4′-galactosyl-kojibiose and lactulosucrose and the potential prebiotics lactosucrose and kojibiose. The novel oligosaccharides were synthesised from equimolar sucrose:lactose and sucrose:lactulose mixtures, respectively, using a *Leuconostoc mesenteroides* dextransucrase and purified by liquid chromatography. The growth of the strains using the purified carbohydrates as the sole carbon source was evaluated by recording the culture optical density and calculating maximum growth rates and lag phase parameters. The results revealed an apparent bifidogenic effect of lactulosucrose, being a moderate substrate for streptococci and not utilised by lactobacilli. In addition, 4′-galactosyl-kojibiose was selectively fermented by *Bifidobacterium breve*, which was the only tested bifidobacterial species able to ferment kojibiose. The described fermentation properties of the specific probiotic strains on the lactose-derived oligosaccharides would enable the design of prebiotics with a high degree of selectivity.

1. Introduction

The human large intestine is densely colonised by a complex microbial ecosystem that plays a key role in human health and disease, with its characterisation being the subject of studies in recent years (Aagaard et al., 2013; Qin et al., 2010). Intestinal bacteria provide a diverse range of biochemical and metabolic activities to complement host physiology and exert numerous protective effects by modulating mucosal and systemic immune responses (Clemente, Ursell, Parfrey, & Knight, 2012). Among the metabolic functions, the fermentation of non-digestible dietary components, which are principally fibre carbohydrates, and the impact on the composition and/or activity of the human gut microbiota are attracting considerable attention in food and nutrition research (Roberfroid et al., 2010).

Prebiotics are normally non-digestible dietary carbohydrates which are selectively fermented, resulting in specific changes in the composition and/or activity of a limited number of intestinal bacteria, thus conferring benefit(s) upon host health (Roberfroid et al., 2010). Currently, there are certain carbohydrates with well-known prebiotic status, and these include lactulose, inulin, fructo-oligosaccharides, galacto-oligosaccharides and resistant starch (Di Bartolomeo, Startek, & Van den Ende, 2013; Slavin, 2013). However, over the last decades there has been a growing search for new prebiotic carbohydrates which could be considered as emerging prebiotics, such as xylo-oligosaccharides, arabinoxylo-oligosaccharides, isomalto-oligosaccharides, lactosucrose and pectic-oligosaccharides, among others (Rastall & Gibson, 2002). Likewise, significant research efforts are currently focused on the search and/or production of novel prebiotic ingredients that have a series of desirable properties including (i) active at low dosage, (ii) lack of side effects, (iii) persistence through the colon, (iv) fine control of microbiota modulation, (v) good storage and processing stability and (vi) possess additional biological activities, exerting beneficial effects on specific physiological functions and/or reducing the risk of disease, for example, through their effect on displacement of pathogens and/or regulation of the function of the immune system (Rastall & Hotchkiss, 2003).

In this context, we have recently described the efficient dextransucrase-catalysed synthesis of oligosaccharides such as lactulosucrose (Díez-Municio, Herrero, Jimeno, Olano, & Moreno, 2012a) and 2-α-glucosyl-lactose, also denominated 4′-galactosyl-kojibiose (Díez-Municio et al., 2012b), whose structural features makes them promising candidates for novel prebiotic ingredients.

* Corresponding author. Tel.: +34 91 0017900.
E-mail address: t.requena@csic.es (T. Requena).

http://dx.doi.org/10.1016/j.idairyj.2014.03.012
0958-6946/

Nevertheless, to the best of our knowledge, no data on selective fermentation by bacteria have been reported for these novel oligosaccharides. In addition, high-yield and high-purity kojibiose has been recently obtained from the complete hydrolysis of 4′-galactosyl-kojibiose by *Kluyveromyces lactis* β-galactosidase (Díez-Municio, Montilla, Moreno, & Herrero, 2014). Kojibiose is a natural disaccharide commercially available only in low amounts due to different difficulties related to its isolation and/or synthesis. Nonetheless, the limited available data have pointed to kojibiose having promising potential as a prebiotic (Sanz, Gibson, & Rastall, 2005).

In this work, the ability of kojibiose and novel lactose-derived oligosaccharides, i.e., 4′-galactosyl-kojibiose and lactulosucrose, to be metabolised by pure cultures of potential probiotic strains belonging to the genera *Lactobacillus*, *Bifidobacterium* and *Streptococcus* is evaluated and compared with well-established (lactulose) or emerging (lactosucrose) lactose-derived prebiotics. Considering that there is a strong link between the oligosaccharide chemical structure and its potential bioactivities, the results derived from this study could provide further insights into the influence of the monomer composition and glycosidic linkage type on the selective fermentability of lactose-derived oligosaccharides by specific probiotic strains.

2. Materials and methods

2.1. Chemicals, reagents, standards and enzymes

All chemicals and reagents used were of analytical grade and purchased from Sigma–Aldrich (St. Louis, MO, USA), VWR (Barcelona, Spain), and Merck (Darmstadt, Germany). Ultra-pure water quality (18.2 MΩcm) with 1–5 ppb total organic carbon (TOC) and <0.001 EU mL^{-1} pyrogen levels was produced in-house using a laboratory water purification Milli-Q Synthesis A10 system from Millipore (Billerica, MA, USA).

Carbohydrates (fructose, glucose, galactose, sucrose, leucrose, lactulose and lactose) were all purchased from Sigma–Aldrich (St. Louis, MO, USA), standard kojibiose was purchased from Carbosynth (Berkshire, UK) and lactosucrose from Wako Pure Chemical Industries (Osaka, Japan).

Dextransucrase from *Leuconostoc mesenteroides* B-512F was purchased from CRITT Bio-Industries (Toulouse, France). Specific activity was 0.4 U mg^{-1}, where 1 U is the amount of enzyme required to perform the transfer of 1 μmol of glucose per minute at a working temperature of 30 °C, a sucrose concentration of 100 g L^{-1} at pH 5.2 in 20 mM sodium acetate buffer, containing 0.34 mM $CaCl_2$. Soluble commercial preparation of β-galactosidase from *K. lactis* (Lactozym Pure 6500 L) was kindly supplied by Novozymes (Bagsvaerd, Denmark).

2.2. Synthesis, purification and characterisation of the studied oligosaccharides

The trisaccharide lactulosucrose, β-D-galactopyranosyl-(1 → 4)-β-D-fructofuranosyl-(2 → 1)-α-D-glucopyranoside, was enzymatically synthesised from sucrose:lactulose mixtures by the *L. mesenteroides* B-512F dextransucrase-catalysed transfer of the glucosyl residue from sucrose to the C-2 of the reducing unit of lactulose as described by Díez-Municio et al. (2012a). The enzymatic reaction was carried out with 30% (w/v) sucrose and 30% (w/v) lactulose and an enzyme charge of 2.4 U mL^{-1} at 30 °C in 20 mM sodium acetate buffer, pH 5.2, containing 0.34 mM $CaCl_2$ for 24 h of reaction time. Under these optimal conditions, lactulosucrose yield was around 30% in weight respect to the initial amount of lactulose.

The enzymatic synthesis of the trisaccharide 4′-galactosyl-kojibiose also termed as 2-α-D-glucopyranosyl-lactose, *O*-β-D-galactopyranosyl-(1 → 4)-*O*-α-D-glucopyranosyl-(2 → 1)-α-D-glucopyranose, was carried out in the presence of sucrose (donor) and lactose as acceptor (30:30, expressed in g 100 mL^{-1}), in 20 mM sodium acetate buffer, pH 5.2, containing 0.34 mM $CaCl_2$ at 30 °C using a *L. mesenteroides* B-512F dextransucrase (0.8 U mL^{-1}) as described by Díez-Municio et al. (2012b). The optimal synthesis conditions at 24 h of enzymatic reaction gave rise to yields close to 50% (in weight compared with the initial amount of lactose).

Kojibiose (2-*O*-α-D-glucopyranosyl-α-D-glucopyranose) was obtained from the hydrolysis of 4′-galactosyl-kojibiose by *K. lactis* β-galactosidase, after removal of residual monosaccharides by using a *Saccharomyces cerevisiae* treatment as described by Díez-Municio et al. (2014).

Isolation of pure oligosaccharides was performed by liquid chromatography with a refractive index detector (LC-RID) on an Agilent Technologies 1260 Infinity LC System (Boeblingen, Germany) using a Zorbax NH_2 PrepHT preparative column (250 × 21.2 mm, 7 μm particle size) (Agilent Technologies, Madrid, Spain). Two millilitres of reaction mixtures (200 mg of total carbohydrates) were eluted with acetonitrile:water as the mobile phase at a flow rate of 21.0 mL min^{-1} for 30 min. The separated oligosaccharides were collected using an Agilent Technologies 1260 Infinity preparative-scale fraction collector (Boeblingen, Germany), and the fractions were pooled, evaporated in a rotatory evaporator R-200 (Büchi Labortechnik AG, Flawil, Switzerland) below 25 °C and freeze-dried. Purity grade was in all cases ≥99.0% as checked by LC-RID.

Lactulosucrose and 4′-galactosyl-kojibiose were then fully characterised by 1D and 2D [^{1}H, ^{1}H] and [^{1}H–^{13}C] nuclear magnetic resonance (NMR) experiments (gCOSY, TOCSY, ROESY, multiplicity-edited gHSQC and gHMBC) (Díez-Municio et al., 2012a, 2012b), whilst kojibiose was identified by LC-RID and gas chromatography with mass spectrometry detection (GC-MS) analyses by comparison with a commercial standard (Díez-Municio et al., 2014).

2.3. Bacterial strains and culture media

Streptococcus salivarius ZL50-7, *Lactobacillus reuteri* R13, *Lactobacillus delbrueckii* ZL95-27, *Bifidobacterium breve* 26M2 and *Bifidobacterium bifidum* HDD541 were isolated from milk of healthy mothers or infant faeces and belong to the culture collection of the Department of Nutrition and Food and Science Technology, Universidad Complutense de Madrid (Madrid, Spain). *Streptococcus thermophilus* STY-31, *Lactobacillus acidophilus* LA-5, *Lactobacillus casei* LC-01 and *Bifidobacterium lactis* BB-12 were isolated from a commercial symbiotic product (Simbiotic Drink, Priégola, Madrid, Spain) as described by Tabasco, Paarup, Janer, Peláez, and Requena (2007). *Lactobacillus rhamnosus* GR-1 is a commercial probiotic strain isolated from the human urogenital tract (Chan, Bruce, & Reid, 1984). Before being used in experiments, all strains were routinely cultured in MRS broth (Pronadisa, Madrid, Spain), except for *S. salivarius* ZL50-7 and *S. thermophilus* STY-31 that were grown in ESTY broth (Pronadisa) containing 20 g L^{-1} lactose (ESTY-L).

Basic media for experiments were MRS fermentation broth (Pronadisa), which does not contain either glucose or meat extract (De Man, Rogosa, & Sharpe, 1960), enriched with 0.2% Tween 80, 0.8% casein acid hydrolysate and 0.05% L-cysteine, or ESTY broth (Pronadisa), without any carbon source. These media were supplemented with the tested oligosaccharides at a final concentration of 0.3% (w/v). Each substrate was weighed and then added to the corresponding autoclaved basic media, and the mixtures were filter sterilised with 0.2 μm pore-size sterile filters. Glucose and lactulose were used as control of growth, being added at the same final