Bioactive Carbohydrates and Dietary Fibre xxx (xxxx) xxx–xxx

Contents lists available at ScienceDirect

Bioactive Carbohydrates and Dietary Fibre

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

In vitro fermentation of beta-glucans and other selected carbohydrates by infant fecal inoculum: An evaluation of their potential as prebiotics in infant formula

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ARTICLE INFO

Keywords:
Beta-glucans
Fecal fermentation
Infant formula
Polysaccharides
Prebiotics

ABSTRACT

Prebiotics are being added to infant formula in order to mimic the effects of human milk oligosaccharides (HMOs) for the growth of probiotic bacteria especially bifidobacteria and lactobacilli in the infant gut. This preliminary study compares the in vitro fermentation of 13 different carbohydrates including monosaccharides, disaccharides, oligosaccharides and polysaccharides by infant fecal samples collected from 3-month old breast-milk fed babies. The growth of the total anaerobic bacteria and two probiotic bacteria (bifidobacteria and lactobacilli) during the fermentation period was measured by total plate count (TPC) and was expressed as colony forming units (CFUs). Among other things, beta-glucans seem to selectively enhance the growth of lactobacilli for a longer period of fermentation time than most of the carbohydrates tested. The selective enrichment of the probiotic bacteria by these carbohydrates and their potential use as prebiotics in the infant formula are discussed.

1. Introduction

The development of innate immune system of infants is strongly dependent on the gut microbiome (Mshvildadze & Neu, 2010) especially during the first 1000 days, which is approximately 3 years. The gut microbiome of infants are mainly affected by the mode of delivery, diet, and whether the infants are breast-fed (Fernández et al., 2013) or formula-fed (Backhed et al., 2015). According to World Health Organization, mothers are encouraged to have their infants breast-fed for 2 years or at least 6 months after birth (http://www.who.int/topics/breastfeeding/en/). Nowadays, most breast-feeding mothers introduce solid food after 6 months of breast-feeding. The first 6 months of child birth is being selected as target of most infant formula products known as stage-one formula in the markets. Strong scientific evidences have shown that there is a significant difference in the gut microbiome profile between babies fed with breast milk that contains human milk oligosaccharides (HMOs) versus babies fed with infant formula, which influences the development of their immune system (Mueller, Bakacs, Combellick, Grigoryan, & Dominguez-Bello, 2015).

In order to mimic the effect of the natural prebiotic HMOs that are present in breast milk, infant formula manufacturers have tried to incorporate prebiotics, probiotics or synbiotics into their products in order to give a similar effect of HMOs on the infant gut microbiome profile (Al-Sheraji et al., 2013; Barile & Rastall, 2013). Common prebiotics used commercially include fructo-oligosaccharides (FOS) and galacto-oligosaccharides (GOS), to be added either individually or combined in different ratios (Candela, Maccaferri, Turroni, Carnevali, & Brigidi, 2010). Lactic acid bacteria (LAB) including bifidobacteria and lactobacilli are commonly added as probiotics (Mueller et al., 2015).

Beta-glucans are well known bioactive carbohydrates with multiple functions and recently it has been proposed to be a potential prebiotics (Lam & Cheung, 2013). Beta-glucans can function as an immunomodulator directly and indirectly via the modulation of gut microbiome (Arena et al., 2016). In this in vitro fermentation study using infant fecal inoculum, beta-glucans isolated from oat, barley, and mushroom (*Pleurotus tuber-regium*) (Zhao & Cheung, 2011) were used to compare with other carbohydrates including monosaccharides, disaccharides and prebiotic oligosaccharides for their potential as prebiotics in terms of selective support for the growth of LAB measured by total plate count (TPC) technique.

2. Materials and methods

All reagents and chemicals were obtained from Sigma Aldrich (USA) unless otherwise specified. Agar made from Reinforced Clostridial

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http://dx.doi.org/10.1016/j.bcdf.2017.07.009
Received 6 July 2017; Received in revised form 25 July 2017; Accepted 26 July 2017

Table 1
Summary of selected carbohydrates used in this study.

Carbon source	Class	Commercial products	Structural characteristic	Purpose of use/other information	Suppliers
Glucose	Simple sugars	NA	α-D-Glucose	Act as a positive control to ensure the viability of bacteria in fecal samples. Not a prebiotic.	Sigma-Aldrich
Lactose		NA	β-D-Galactose-(1→4)-α-D-Glucose	The most abundant carbohydrate in breast milk, account to over 90% of total carbohydrate with the rest 10% being glycomes (HMOs).	Sigma-Aldrich
Sucrose		NA	α-D-Glucose-(1→2)-β-D-Fructose	Added in infant formula to give sweetness. Limited prebiotic property.	Sigma-Aldrich
Xylitol	Sugar alcohol	NA	Xylitol	A sugar alcohol sweetener that can be absorbed in the human gut. Produced by partial fermentation from hemicellulose hydrolysate	Wako Pure Chemical Industries, Ltd
FOS	Oligo-saccharides	Cow & Gate, Illuma, Physiolac, Wyeth, Meiji, Earth Best Organic	Glucose-(Fructose)$_n$ with β−2→1 linkage between the fructose monomer units. Average degree of polymerization > 10	Mixtures of 1-ketose, nystose, & 1-fructofluranosyl-D-nystose. Most common prebiotics used in infant formula. They are resistant to hydrolysis by the mammalian intestinal enzymes but are fermented extensively by colonic microflora and promote the growth of LAB	Wako Pure Chemical Industries, Ltd
GOS		Cow & Gate, Snow Brand, Mead Johnson, Abbott, Friso, Physiolac, Glico Group, Maeil, Wakodo, Morinaga	Mixture of β−1,4-linked D-galacto-oligosaccharides, galactose, glucose and lactose	A functional sweetener that contains 55% or more galacto-oligosaccharide and is manufactured by the action of enzymes on lactose. Most common prebiotics used in infant formula. They are resistant to hydrolysis by the mammalian intestinal enzymes but are fermented extensively by colonic microflora and promote the growth of LAB	Oligomate, Yakult Pharmaceutical Industry Co., Ltd
XOS		NA	β−1,4-linked D-Xylose	Potential prebiotics. Industrially produced by treating xylans with xylanase.	Wako Pure Chemical Industries, Ltd
Oat_bG	Beta-glucans	NA	β−1,3 and 1,4-linked D-Glucose	A highly water soluble beta-glucan with a linear structure of MW 18 kDa.	Xi'an Rongsheng
Ptr_bG		NA	β−1,3 and 1,4-linked D-Glucose with β−1,6-D-Glucose branching	A gel forming highly branched beta-glucan with MW of 96 kDa. An alkali-soluble extract from the sclerotia of *P. tuber-regium*	Own preparation
Barley_bG		NA	β−1,3 and 1,4-linked D-Glucose	A low viscosity beta-glucan with a linear structure, MW of 179 kDa, viscosity of 11 cSt	Megazyme
Glycogen	Alpha-glucans	NA	α−1,4-linked D-Glucose with α−1,6 Glucose branch	Ultrapure soluble glycogen from oyster which can be hydrolyzed by human digestive enzymes.	Affymetrix, USB
Starch		NA	α−1,4-linked D-Glucose with α−1,6 Glucose branching	Soluble starch from potato which can be hydrolyzed by human digestive enzymes.	Sigma-Aldrich
Inulin	Long chain FOS	NA	Glucose-(Fructose)$_n$ with β−2→1 linkage between the fructose monomer units.	Inulin extracted from Dahlia Tubers with Mw ~5 kDa. Less readily fermented by colonic bacteria as compared to FOS.	Sigma-Aldrich

NA – not applicable.