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Carbohydrate catabolic diversity of bifidobacteria and lactobacilli of human origin

Heather P. McLaughlin [a,d], Mary O'Connell Motherway [a,d], Bhuvaneswari Lakshminarayanan [b,d], Catherine Stanton [b,d], R. Paul Ross [b,d], Jennifer Brulc [c], Ravi Menon [c], Paul W. O'Toole [a,d], Douwe van Sinderen [a,d,*]

[a] School of Microbiology, University College Cork, Cork, Ireland
[b] Teagasc Moorepark, Fermoy, Co. Cork, Ireland
[c] General Mills, Bell Institute of Health & Nutrition, Minneapolis, MN, USA
[d] Alimentary Pharmabiotic Centre, Cork, Ireland

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ABSTRACT

Because increased proportions of particular commensal bacteria such as bifidobacteria and lactobacilli have been linked to human health through a variety of mechanisms, there is corresponding interest in identifying carbohydrates that promote growth and metabolic activity of these bacteria. We evaluated the ability of 20 carbohydrates, including several commercially available carbohydrates that are sold as prebiotic ingredients, to support growth of 32 human-derived isolates belonging to the genera *Bifidobacterium* and *Lactobacillus*, including those isolated from healthy elderly subjects. In general, bifidobacterial strains were shown to display more diverse carbohydrate utilization profiles compared to the tested *Lactobacillus* species, with several bifidobacterial strains capable of metabolizing xylo-oligosaccharide (XOS), arabinoxylan, maltodextrin, galactan and carbohydrates containing fructo-oligosaccharide (FOS) components. In contrast, maltodextrin, galactan, arabinogalactan and galactomannan did not support robust growth ($\geq$0.8 $OD_{600\ nm}$) of any of the *Lactobacillus* strains assessed. Carbohydrate fermentation was variable among strains tested of the same species for both genera. This study advances our knowledge of polysaccharide utilization by human gut commensals, and provides information for the rational design of selective prebiotic food ingredients.

1. Introduction

Improving health and/or reducing the threat of chronic disease are some of the forces driving the development of functional foods for humans and animals. There is both scientific and commercial interest in the concept of prebiotics which aim to beneficially modulate gut microbiota composition and associated bacterial metabolic activities. Bifidobacteria and lactobacilli are often targeted for prebiotic intervention as they have been reported to confer various health benefits, including, but not limited to, immune-modulation (Konstantinov et al., 2008; Medina et al., 2007; Turroni et al., 2014), restriction of pathogenic bacteria through competition and production of short chain fatty acids (SCFAs) (Schiffrin and Blum, 2002; Servin, 2004; Ventura et al., 2012) and modulation of mucosal barrier function (Miyauchi et al., 2012; Turroni et al., 2014; Yan et al., 2007; Yan and Polk, 2002).

While carbohydrates such as fructose commonly consumed in the Western diet do not fulfill the definition of a prebiotic (Gibson et al., 2004; Johnson et al., 2007), for instance resistance to host digestion, fermentation by gut microbiota and selective stimulation of growth and/or metabolic activity of health-promoting bacteria (Roberfroid, 2007), significant efforts are being made to identify novel compounds with prebiotic potential and to expand our knowledge on those that do fulfill this prebiotic concept. To date, carbohydrates derived from various sources such as arabinoxylan, resistant starch, β-glucan, galacto-oligosaccharides (GOS), fructo-oligosaccharides (FOS), xylo-oligosaccharides (XOS) and inulin have demonstrated prebiotic effects (Broekaert et al., 2011; Kelly, 2008; Macfarlane et al., 2008; Zhao and Cheung, 2011). For instance, it has been shown that many *Bifidobacterium* strains are capable of fermenting FOS, whereas only a minority are able to grow on inulin, although both FOS and inulin proved bifidogenic, presumably as a result of cross-feeding in fecal cultures (Rossi et al., 2005). Carbohydrate-dependent short-chain fatty acid production was also observed in this study where butyrate was the major fermentation product on inulin, while lactate and acetate were produced on FOS (Rossi et al., 2005). Arabinoxylan- and xylo-oligosaccharides,

* Corresponding author at: Room 4.05, Biosciences Institute University College Cork, Cork, Ireland, Tel.: +353 21 490 1365; fax: +353 21 490 3101.
E-mail address: d.vansinderen@ucc.ie (D. van Sinderen).

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which have been shown to resist degradation under conditions mimicking the human stomach (Courtin et al., 2009), were completely fermented by fecal microbiota after 80 h of *in vitro* cultivation (Kabel et al., 2002), and were utilized by *Bifidobacterium* and *Lactobacillus* strains when grown in pure culture (Moura et al., 2007). Synergistic effects have also been reported for the consumption of more than one prebiotic at the same time, for example in rats administered a combination of FOS and resistant starch (Rodriguez-Cabezas et al., 2010). Convincing data has also been reported for the prebiotic effects of certain carbohydrates, such as FOS, GOS, and inulin, based on their inclusion in human clinical trials (Davis et al., 2010; Kolida et al., 2007; Lindsay et al., 2006).

While bifidobacteria and lactobacilli are usually targeted as potential probiotic bacteria resident in the human intestine, these bacteria differ in their fermentation abilities and molecular mechanisms by which they consume carbohydrates. In comparative studies, it has been reported that growth of bifidobacterial strains is supported by a larger number of test carbohydrates than lactobacilli (Watson et al., 2013). Based on annotations, more than 8% of the bifidobacterial genome is devoted to carbohydrate metabolism which may explain their more versatile carbohydrate utilization profiles (Kim et al., 2009; Pokusaeva et al., 2011; Schell et al., 2002; Sela et al., 2008). In addition, shifts in endogenous microbial communities in the gut have been observed as a result of both dietary changes and age category (Claesson et al., 2012; Muegge et al., 2011). Data suggests that species such as *Bifidobacterium longum*, *Bifidobacterium adolescentis*, *Lactobacillus rhamnosus* and *Lactobacillus casei/paracasei* are more predominant in adults, whereas *Bifidobacterium breve*, *B. longum* subsp. *infantis*, *Lactobacillus gasseri* and *Lactobacillus salivarius* are more commonly isolated from the infant gut (Reuter, 2001; Wall et al., 2007). Because the relative abundance and prevalence of particular intestinal probiotic species is known to fluctuate, strains may have adapted varying carbohydrate utilization strategies and an understanding of these differences is imperative when using a rational approach for the formulation of functional foods that include prebiotic substrates. Studies which investigate individual strains from these commensal groups are also relevant on the basis that they are not considered probiotics, by definition, until health-promoting characteristics are clearly and clinically demonstrated (Kleerebezem and Vaughan, 2009).

In the current study, pure culture *in vitro* experiments were conducted to investigate the ability of 20 carbohydrates, including several commercially available prebiotics, to support the growth of 32 human-derived isolates from various individuals of different age profiles, and belonging to the genera *Bifidobacterium* and *Lactobacillus*. This study advances the knowledge of carbohydrate utilization by these strains by demonstrating selective stimulation of growth and/or activity on a wide range of carbohydrates, as well as by generating significant data on both inter- and intra-species diversity in relation to growth abilities and consumption of specific carbohydrate components.

2. Materials and methods

2.1. Bacterial strains and culture conditions

Thirty two human-derived bifidobacteria and lactobacilli were included in this study (Table 1), including mostly intestinal isolates and representing members of nine species for each genus. This set included 11 strains that had previously been isolated from elderly volunteers as part of the ELDERMET project (Claesson et al., 2011), while the remaining strains originated from infants or adults. All *Bifidobacterium* and *Lactobacillus* strains were routinely cultured anaerobically at 37 °C in Reinforced Clostridium Medium (RCM) (Oxoid, Hampshire, England) or Lactobacilli de Man-Rogosa and Sharpe (LMRS) medium (Difco, France), respectively. For solid medium, 1.5% (w/v) agar (Oxoid, Basingstoke, UK) was added. For evaluation of growth on carbohydrates, strains were inoculated into modified MRS (mMRS) medium (de Mann et al., 1960) prepared from first principle, and comprising of trypticase peptone (10.0 g/L), granulated yeast extract (2.5 g/L), tryptose (3.0 g/L), K_2HPO_4 (3.0 g/L), KH_2PO_4 (3.0 g/L), tri-ammonium citrate (2.0 g/L), pyruvic acid (0.2 g/L), cysteine-HCl (0.3 g/L), Tween-80 (1 mL), $MgSO_4 \cdot 7H_2O$ (0.575 g/L), $MnSO_4 \cdot 4H_2O$ (0.12 g/L), and $FeSO_4 \cdot 7H_2O$ (0.034 g/L). Prior to autoclaving, mMRS medium was adjusted to pH 6.8. All strains were stocked in appropriate medium with the addition of 80% glycerol and stored at −80 °C.

2.2. Carbohydrate solutions

Glucose and lactose (Sigma-Aldrich, Steinheim, Germany) served as control carbohydrates for bacterial growth studies. The oligo- and polysaccharides included in this study are detailed in Table 2. Solutions of the carbohydrates to be tested including maltodextrin, Beneo Orafti HSI and GR, Sensus Frutafit CLR, Roquette Nutriose FM06 and FB06, Tate & Lyle Promitor SCF 85, ADM Fibersol-2 SCF, Ciranda organic inulin and xylo-oligosaccharides were prepared by dissolution at 5% (w/v) final concentration in distilled water followed by filter-sterilization using Minisart filters (0.45 μm pore size, Sartorius AG, Gottingen,

Table 1
Bacterial strains used in this study.

Strain	Isolated from	Strain	Isolated from
B. adolescentis CIP64.61	Human adult intestine	*L. acidophilus* ATCC 4356	Human
B. adolescentis NCFB 2229	Human adult intestine	*L. acidophilus* NCFM	Human
B. animalis ssp. *lactis* EM109-6 (EM)	Human elderly feces	*L. amylovorus* DSM20052	Human adult intestine
B. animalis ssp. *lactis* EM051-3 (EM)	Human elderly feces	*L. casei* EM100-11 (EM)	Human elderly feces
B. animalis ssp. *lactis* EM094-6 (EM)	Human elderly feces	*L. delbrueckii* ssp. *lactis* DSM20073	Saliva
B. bifidum NCIMB 8810	Human	L. *kalixensis* DSM 16043	Human stomach mucosa
B. breve JCM7017	Human feces	*L. oris* DSM 4864	Human saliva
B. breve JCM7019	Human infant feces	*L. parabuchneri* DSM 5707	Human saliva
B. breve UCC2003	Human infant feces	*L. plantarum* NCDO326	Human dental carries
B. dentium NCFB 2843	Human dental carries	*L. reuteri* ATCC 55730	Human feces
B. infantis NCDO 2205	Human infant intestine		
B. lactis Bb1 (Nestle)	Human infant feces		
B. longum infantis CCUG18157	Human feces		
B. longum NCIMB 8809	Human feces		
B. longum EM223 T0-M3 (EM)	Human elderly feces		
B. longum EM044 T0-1 (EM)	Human elderly feces		
B. longum EM193 T3-1 (EM)	Human elderly feces		
B. longum EM049 T0-6 (EM)	Human elderly feces		
B. longum EM176 T0-1 (EM)	Human elderly feces		
B. pseudocatenulatum NCIMB 8811	Human infant feces		
Bifidobacterium EM193 T0-1 (EM)	Human elderly feces		
Bifidobacterium EM181 T3-1 (EM)	Human elderly feces		