International Journal of Food Microbiology 231 (2016) 76–85

Contents lists available at ScienceDirect

International Journal of Food Microbiology

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

Bifidobacterial inulin-type fructan degradation capacity determines cross-feeding interactions between bifidobacteria and *Faecalibacterium prausnitzii*

Frédéric Moens, Stefan Weckx, Luc De Vuyst *

Research Group of Industrial Microbiology and Food Biotechnology, Faculty of Sciences and Bioengineering Sciences, Vrije Universiteit Brussel, Pleinlaan 2, B-1050 Brussels, Belgium

ARTICLE INFO

Article history:
Received 5 December 2015
Received in revised form 31 March 2016
Accepted 8 May 2016
Available online 12 May 2016

Keywords:
Inulin-type fructans
Cross-feeding
Commensalism
Competition

ABSTRACT

Prebiotic inulin-type fructans (ITF) display a bifidogenic and butyrogenic effect. Four bifidobacterial strains (*Bifidobacterium breve* Yakult, *Bifidobacterium adolescentis* LMG 10734, *Bifidobacterium angulatum* LMG 11039^T, and *Bifidobacterium longum* subsp. *longum* LMG 11047), displaying different ITF degradation capacities, were each grown in cocultivation with *Faecalibacterium prausnitzii* DSM 17677^T, an ITF-degrading butyrate-producing colon bacterium, as to unravel their cross-feeding interactions. These coculture fermentations were performed in a medium for colon bacteria, whether or not including acetate (necessary for the growth of *F. prausnitzii* DSM 17677^T and whether or not provided through cross-feeding), supplemented with oligofructose or inulin as the sole energy source. *Bifidobacterium breve* Yakult did not degrade oligofructose, resulting in the production of high concentrations of butyrate by *F. prausnitzii* DSM 17677^T through oligofructose degradation. The degradation of oligofructose by *B. adolescentis* LMG 10734 and of oligofructose and inulin by *B. angulatum* LMG 11039^T and *B. longum* LMG 11047 resulted in the production of acetate, which was cross-fed to *F. prausnitzii* DSM 17677^T, enabling the latter strain to degrade oligofructose and inulin. Slow preferential degradation of the short chain length fractions of oligofructose (intracellularly) by *B. adolescentis* LMG 10734 enabled substantial oligofructose degradation by *F. prausnitzii* DSM 17677^T. However, fast non-preferential degradation of all chain length fractions of oligofructose (extracellularly) and efficient degradation of the short chain length fractions of inulin by *B. angulatum* LMG 11039^T and *B. longum* LMG 11047 made it impossible for *F. prausnitzii* DSM 17677^T to compete for the available substrate. These results indicate that cross-feeding interactions between bifidobacteria and acetate-depending, butyrate-producing colon bacteria can be either a pure commensal or beneficial relationship between these bacteria, or can be dominated by competition, depending on the ITF degradation capacities of the bifidobacterial strains involved.

1. Introduction

The prebiotic effect of inulin-type fructans (ITF), which are linear polymers consisting of β-2,1-linked fructose monomers, often with a terminal glucose moiety, was originally defined as the selective stimulation of the growth and/or activity of bifidobacteria in the human colon (Gibson and Roberfroid, 1995). This bifidogenic effect has been shown repeatedly both *in vitro* (Falony et al., 2009a; Rossi et al., 2005) and *in vivo* (De Preter et al., 2008; Dewulf et al., 2013; Joossens et al., 2012; Ramirez-Farias et al., 2009). However, this stimulation is strongly species- and even strain-dependent (Dewulf et al., 2013; Falony et al., 2009a; Joossens et al., 2012; Ramirez-Farias et al., 2009; Rossi et al., 2005). In this context, various ITF degradation fingerprints have been distinguished among bifidobacterial strains, *i.e.*, their capacity to degrade fructose, oligofructose (various chain length fractions, either consecutively or simultaneously), and inulin (short and/or long chain length fractions). As a consequence, 16 bifidobacterial strains could be subdivided into four different clusters A–D (Falony et al., 2009a). Cluster A bifidobacteria degrade neither oligofructose nor inulin, cluster B bifidobacteria are incapable to degrade inulin but do degrade oligofructose with a preference towards the short chain length fractions, and cluster C and D bifidobacteria degrade oligofructose in a non-preferential way and are capable of partial inulin degradation. Besides bifidobacteria other beneficial colon bacteria are able to degrade prebiotic ITF. This is the

* Corresponding author at: Research Group of Industrial Microbiology and Food Biotechnology (IMDO), Vrije Universiteit Brussel (VUB), Pleinlaan 2, B-1050 Brussels, Belgium.
E-mail address: ldvuyst@vub.ac.be (L. De Vuyst).

http://dx.doi.org/10.1016/j.ijfoodmicro.2016.05.015
0168-1605/

case for lactobacilli (Makras et al., 2005) and some butyrate-producing colon bacteria such as *Roseburia* spp., *Eubacterium rectale*, and *Faecalibacterium prausnitzii* (Falony et al., 2009b; Moens et al., 2014b).

Faecalibacterium prausnitzii is a butyrate-producing colon bacterium belonging to *Clostridium* cluster IV and represents up to 8% of the microbiota of humans consuming a Western-type diet (Walker et al., 2011). Two *in vivo* studies have demonstrated that this bacterium can be significantly stimulated upon ITF administration (Dewulf et al., 2013; Ramirez-Farias et al., 2009). Furthermore, it has been shown that the type strain *F. prausnitzii* DSM 17677^T performs an extracellular degradation of oligofructose and is able to degrade the long chain length fractions of inulin, provided acetate is present in the culture medium (Moens et al., 2014b). This ITF degradation pattern and acetate dependence is also associated with ITF degradation by *Roseburia inulinivorans* DSM 16841^T (Falony et al., 2009b) and *E. rectale* CIP 105953^T (Moens et al., 2014b).

The butyrogenic effect or stimulation of butyrate-producing colon bacteria and, hence, colon butyrate production by consumption of ITF was first observed during rat and *in vitro* studies (Campbell et al., 1996; Le Blay et al., 1999; Morrison et al., 2006). This activity is of major importance for colon health, since butyrate is the preferred energy source for the colon epithelial cells, displays anti-inflammatory properties, and prevents colon carcinogenesis (Hamer et al., 2008). Also, growth stimulation of butyrate-producing colon bacteria may be beneficial, since several studies have shown that *F. prausnitzii* and *Butyricicoccus pullicaecorum* are represented in low numbers among the colon microbiota of people suffering from inflammatory bowel disease (Eeckhaut et al., 2013; Frank et al., 2007; Jia et al., 2010; Joossens et al., 2011; Lozupone et al., 2012). In particular, *F. prausnitzii* DSM 17677^T is a very promising strain to be used as probiotic, given its anti-inflammatory properties in colitis-induced rats (Miquel et al., 2015; Sokol et al., 2008). A synbiotic formulation, encompassing this highly oxygen-sensitive strain in combination with the well-studied prebiotic inulin, has been shown to guarantee the survival of this strain under aerobic conditions (Khan et al., 2014).

Whereas the combined bifidogenic and butyrogenic effects of ITF were poorly understood in the past, several studies have demonstrated that two types of cross-feeding interactions between bifidobacteria and butyrate-producing colon bacteria are responsible for their simultaneous occurrence (De Vuyst and Leroy, 2011; De Vuyst et al., 2014). Type one cross-feeding interactions occur between oligofructose-degrading bifidobacteria and butyrate-producing colon bacteria that are not capable of oligofructose degradation (Belenguer et al., 2006; Falony et al., 2006). Oligofructose degradation by the bifidobacteria results in the production of acetate and the release of fructose from oligofructose, which are cross-fed to the oligofructose non-degrading, butyrate-producing colon bacteria. Type two cross-feeding interactions occur between oligofructose-degrading bifidobacteria and oligofructose-degrading, acetate-dependent, butyrate-producing colon bacteria (Falony et al., 2006). Oligofructose is initially degraded by the bifidobacteria, which results in the production of acetate that is in turn cross-fed to the butyrate-producing colon bacteria, allowing the latter to degrade oligofructose too. Whereas acetate is a stimulating bifidobacterial metabolite for butyrate-producing colon bacteria during these cross-feeding interactions, it is likely that competition for the available energy sources (ITF or fructose elaborated during ITF degradation) can occur between these bacteria. Consequently, this could result in the production of only minor concentrations of butyrate, which may be disadvantageous in the colon (De Vuyst et al., 2014). Recently, it has been shown that enhanced butyrate production by *F. prausnitzii* S3/L3 and *F. prausnitzii* A2-165 has been attributed to cross-feeding interactions with *Bifidobacterium adolescentis* L2-32 when grown on oligofructose and starch, respectively (El-Semman et al., 2014; Rios-Covian et al., 2015). Given the ITF degradation capacity of *F. prausnitzii* DSM 17677^T (Moens et al., 2014b) and the emergent evidence of its potential beneficial anti-inflammatory properties in diseased mammals (Miquel et al., 2015; Sokol et al., 2008), knowledge on its butyrate production through cross-feeding is necessary, as this may result in commensal interactions with bifidobacteria whether or not accompanied with competition.

The present study aimed at investigating cross-feeding interactions between bifidobacterial strains with different ITF degradation capacities and the ITF-degrading, butyrate-producing colon bacterial strain *F. prausnitzii* DSM 17677^T.

2. Materials and methods

2.1. Microorganisms and media

Faecalibacterium prausnitzii DSM 17677^T was obtained from the Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ, Göttingen, Germany). *Bifidobacterium breve* Yakult (cluster A) was kindly provided by Yakult Honsha Co. Ltd. (Tokyo, Japan). *Bifidobacterium adolescentis* LMG 10734 (cluster B), *Bifidobacterium angulatum* LMG 11039^T (cluster C), and *Bifidobacterium longum* subsp. *longum* LMG 11047 (cluster D) were obtained from the Belgian Coordinated Collections of Microorganisms - Laboratory for Microbiology Ghent (BCCM-LMG, Ghent, Belgium). The selection of the bifidobacterial strains to perform coculture fermentations with *F. prausnitzii* DSM 17677^T was based on the fact that each strain represents a distinct cluster of bifidobacteria, exhibiting different ITF degradation fingerprints (Falony et al., 2009a). All strains used in this study are human isolates and were stored at −80 °C in reinforced clostridial medium (RCM; Oxoid Ltd., Basingstoke, United Kingdom), supplemented with 25% (vol/vol) of glycerol as a cryoprotectant.

A modified version of a medium for colon bacteria (mMCB) to allow growth of *F. prausnitzii* was used throughout this study (Moens, F. & De Vuyst, L., unpublished results). This medium had the following composition (concentrations in g L^{-1}): bacteriological peptone (Oxoid), 6.5; soy peptone (Oxoid), 5.0; yeast extract (VWR International, Darmstadt, Germany), 3.0; tryptone (Oxoid), 2.5; NaCl (VWR International), 4.5; K_2HPO_4 (Merck), 0.45; KH_2PO_4 (Merck), 0.45; $MgSO_4 \cdot 7H_2O$ (Merck), 0.09; $CaCl_2 \cdot 2H_2O$ (Merck), 0.09; cysteine-HCl (Merck), 0.4; $NaHCO_3$ (VWR International), 0.2; $MnSO_4 \cdot H_2O$ (VWR International), 0.05; $FeSO_4 \cdot 7H_2O$ (Merck), 0.005; $ZnSO_4 \cdot 7H_2O$ (VWR International), 0.005; hemin (Sigma-Aldrich, Steinheim, Germany), 0.005; menadione (Sigma-Aldrich), 0.005; and resazurin (Sigma-Aldrich), 0.001. Acetate (50 mM or 6.8 g L^{-1} of $CH_3COO^-Na^+ \cdot 3H_2O$; Merck, Darmstadt, Germany) was added to mMCB for the coculture fermentations with *B. breve* Yakult and *F. prausnitzii* DSM 17677^T to enable the latter strain to grow from the start of the fermentations because of low acetate production by the former strain. The pH of the medium was adjusted to 6.3, mimicking the average pH of the colon, and the medium was autoclaved at 210 kPa and 121 °C for 20 min. After sterilization, oligofructose (Raftilose P95; Beneo-Orafti NV, Tienen, Belgium; Falony et al., 2009a) or inulin (OraftiHP; Beneo-Orafti; Falony et al., 2009a) was added as the sole energy source aseptically, always at a final concentration of 50 mM fructose equivalents (FE), using sterile stock solutions. Oligofructose and inulin stock solutions were sterilized by membrane filtration using Minisart filters (pore size, 0.2 μm; Sartorius, Göttingen, Germany).

2.2. Coculture fermentation experiments

Coculture fermentations were carried out in 2-L Biostat B-DCU fermentors (Sartorius, Melsungen, Germany) containing 1.5 L of mMCB supplemented with the energy source under study. Inocula of the strains were prepared as follows. Cells were transferred from −80 °C into test tubes containing 10 mL of RCM (bifidobacterial strains) or glass bottles containing 100 mL of RCM (*F. prausnitzii* DSM 17677^T), and incubated anaerobically at 37 °C for 12 h (bifidobacterial strains) or 24 h (*F. prausnitzii* DSM 17677^T). Subsequently, the strains were