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Cross-feeding between bifidobacteria and butyrate-producing colon bacteria explains bifdobacterial competitiveness, butyrate production, and gas production

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ABSTRACT

Inulin-type fructans are not digested and reach the human colon intact, where they are selectively fermented by the colon microbiota, in particular bifidobacteria. As a result, they are converted, directly or indirectly, to short-chain fatty acids and other organic acids, as well as gases, and lead to both bifidogenic and butyrogenic health-promoting effects. Bifidobacteria display phenotypic variation on strain level as to their capacity to degrade inulin-type fructans. Also, different chain lengths of inulin-type fructans may stimulate different subgroups within the bifidobacterial population. The end-metabolites of inulin-type fructan degradation by bifidobacteria reflect their growth rates on these polymers. Other colon bacteria are also able to degrade inulin-type fructans, as is the case for lactobacilli, *Bacteroides*, certain enterobacteria, and butyrate producers. Bacterial cross-feeding mechanisms in the colon lay at the basis of overall butyrate production, a functional characteristic of several colon bacteria that is always accompanied by gas production. Finally, specificity of polysaccharide use by the colon microbiota may determine diet-induced alterations in the microbiota and consequent metabolic effects.

1. Introduction

Prebiotics are non-digestible food ingredients that beneficially affect the consumer by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon (De Vuyst et al., 2004; Falony and De Vuyst, 2009; Gibson and Roberfroid, 1995; Gibson et al., 2004). Examples of industrially important prebiotics are inulin-type fructans and galactooligosaccharides (Gibson et al., 2004). Inulin-type fructans largely remain the most studied and well-established prebiotics (Bosscher et al., 2006; Roberfroid and Wolinsky, 2005). They are naturally present in onions, garlic, leek, bananas, and chicory. On an industrial scale, inulin-type fructans are usually extracted from chicory roots. Inulin-type fructans are linear *D*-fructose (F) polymers linked by β(2-1)-glycosidic bonds (F_m-type), often with a terminal glucose (G) moiety that is linked by an α(1-2)-glycosidic bond (GF_n-type), as in sucrose (Fig. 1). Inulin-type fructans encompass short and long polymers of fructose, which is reflected in their degree of polymerization (DP, the value of m and n), further referred to as oligofructose (DP of 2–8, average DP of 4) and long-chain inulin (DP of 12–65, average DP of 25), respectively (Fig. 1). Native inulin (DP of 2–65, average DP of 10) consists of short fractions (short-chain inulin or oligofructose) and long fractions (long-chain inulin) (Fig. 1). Because of their β-linkages, inulin-type fructans are not digested neither are they absorbed in the human upper gastrointestinal tract (Molis et al., 1996). They reach the colon virtually intact, where they are selectively fermented by the colon microbiota, in particular the bifidobacterial communities (Roberfroid et al., 1998). During the complex colon fermentation process, they are mainly converted, directly or indirectly, not only to short-chain fatty acids (SCFAs), such as acetate, propionate, and butyrate, but also to other organic acids (e.g., lactate and succinate) and gases (hydrogen gas and carbon dioxide) (Alles et al., 1996). An important outcome of colon fermentation of inulin-type fructans is an increase in bifidobacterial counts, the so-called bifidogenic effect (Gibson et al., 1995; Roberfroid et al., 1998), as well as an enhancement of colon butyrate production, the so-called butyrogenic effect (Campbell et al., 1997; Falony and De Vuyst, 2009; Le Blay et al., 1999; Morrison et al., 2006; Tsukahara et al., 2003). These bifidogenic and butyrogenic effects are considered beneficial for the consumers' health, but their microbial background was unclear until recently, as bifidobacteria are not able to produce butyrate (Falony and De Vuyst, 2009; Falony et al., 2006; Makras et al., 2006; Van der Meulen et al., 2004). Bifidobacteria are considered to contribute to microbial gut balancing, while butyrate is an important energy source for the colon epithelial cells and may play a role in the development of and the gene expression in intestinal cells, hence possibly preventing colitis and colorectal cancer (Falony and De Vuyst, 2009; Hamer et al., 2008; Macfarlane and Cummings, 1991; Scheppach and Weiler, 2004).

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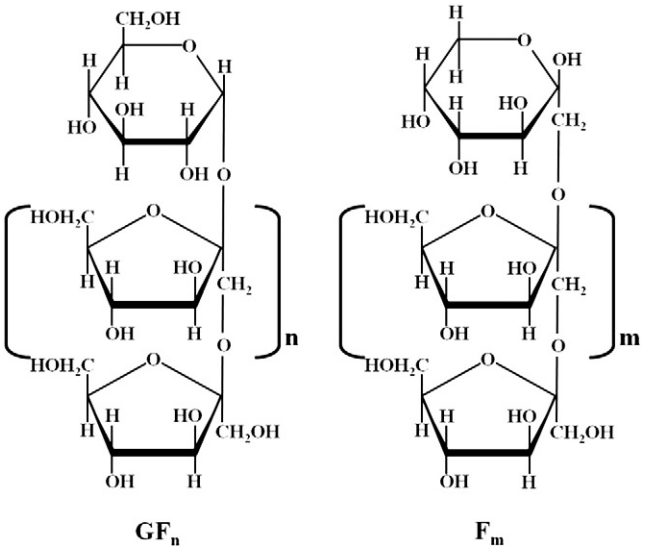

Fig. 1. Chemical structure of inulin-type fructans. F, fructose; G, glucose.

2. Bifidobacteria show a wide phenotypic variation as to their capacity to degrade inulin-type fructans

It is known for a long time that bifidobacteria are capable to degrade inulin-type fructans. At the origin of the development of the prebiotic concept lies the observation of the stimulatory effect of inulin-type fructans on the *Bifidobacterium* population during *in vitro* experiments (Gibson and Roberfroid, 1995; Gibson and Wang, 1994; Gibson et al., 1995; Roberfroid et al., 1998; Wang and Gibson, 1993), which was afterwards largely confirmed by *in vivo* trials (Roberfroid, 2005). Later, the capacity of bifidobacteria to degrade inulin-type fructans has been ascribed to the expression of various β-fructofuranosidase genes, encompassing invertase-, β-fructosidase-, and inulinase-type enzyme activities (Ehrmann et al., 2003; Janer et al., 2004; Kullin et al., 2006; Muramatsu et al., 1993, 1994; Omori et al., 2010; Ryan et al., 2005; Warchol et al., 2002), and the presence of cellular oligosaccharide uptake systems (Flint et al., 2008; Klijn et al., 2005; Parche et al., 2007; Schell et al., 2002). This makes it difficult to precisely link genotype to phenotype without further detailed substrate/enzyme characterization (Kullin et al., 2006; Van den Broek et al., 2008). Furthermore, reports on the degradation of inulin of a high DP are sometimes contradictory. For instance, Rossi et al. (2005) report on the partial degradation of inulin by a majority of bifidobacterial strains and one strain (*Bifidobacterium adolescentis* ALB 1) capable to degrade all fractions, whereas Falony et al. (2009a,c) indicate an upper limit of the DP of about 20 concerning long-chain inulin degradation (Fig. 2C). The latter observation can be supported by earlier findings of low bifidobacterial β-fructofuranosidase activity toward long-chain inulin molecules (Janer et al., 2004; Louis et al., 2007b; Ryan et al., 2005). Hence, it seems likely that not all bifidobacterial species benefit in the same way from the presence of inulin-type fructans as energy sources in the colon. However, in the past, most studies concerning the bifidogenic effect of inulin-type fructans have considered the bifidobacterial colon population as a whole, not taking into account the interspecies differences that exist between various bifidobacteria (Macfarlane et al., 2006). In contrast, statistical analysis of data of a comparative study carried out on eighteen different bifidobacterial strains belonging to various species through characterization of their substrate degradation fingerprints for growth on fructose, oligofructose, and long-chain inulin has revealed the existence of four different groups among the bifidobacterial strains studied (Falony et al., 2009a). However, as strains of the same species belong to different groups, no species specificity regarding the bifidogenic effect of inulin-type fructans could be indicated. Bifidobacterial strains belonging to a first group (group A—e.g., *B. bifidum* LMG 11583) are not capable to degrade either oligofructose or inulin. Bifidobacterial strains categorized in a second group (group B—e.g., *B. adolescentis* LMG 10734) degrade oligofructose, thereby consuming short chains faster than long chains, which has been indicated as a preferential degradation mechanism (Fig. 2A). This preferential degradation mechanism has been studied further in detail and probably is on the basis of the bifidogenic effect (see below). Notice that these group B bifidobacterial strains do not grow on long-chain inulin and that their fructose consumption is faster than their oligofructose degradation. A third group (group C—e.g., *B. longum* LMG 11570) harbors bifidobacterial strains that consume all chain lengths simultaneously and that are even able to degrade long-chain inulin partially (Fig. 2B,C). Although degradation of oligofructose occurs faster than fructose consumption in these strains, this non-preferential degradation mechanism comes as a surprise for bifidobacteria, as it will compromise their competitiveness (see below). Non-preferential oligofructose degradation has been shown unequivocally in the case of colon bacteria other than bifidobacteria (Falony et al., 2009b,c; Makras et al., 2005; Van der Meulen et al., 2006b). A fourth, small group (group

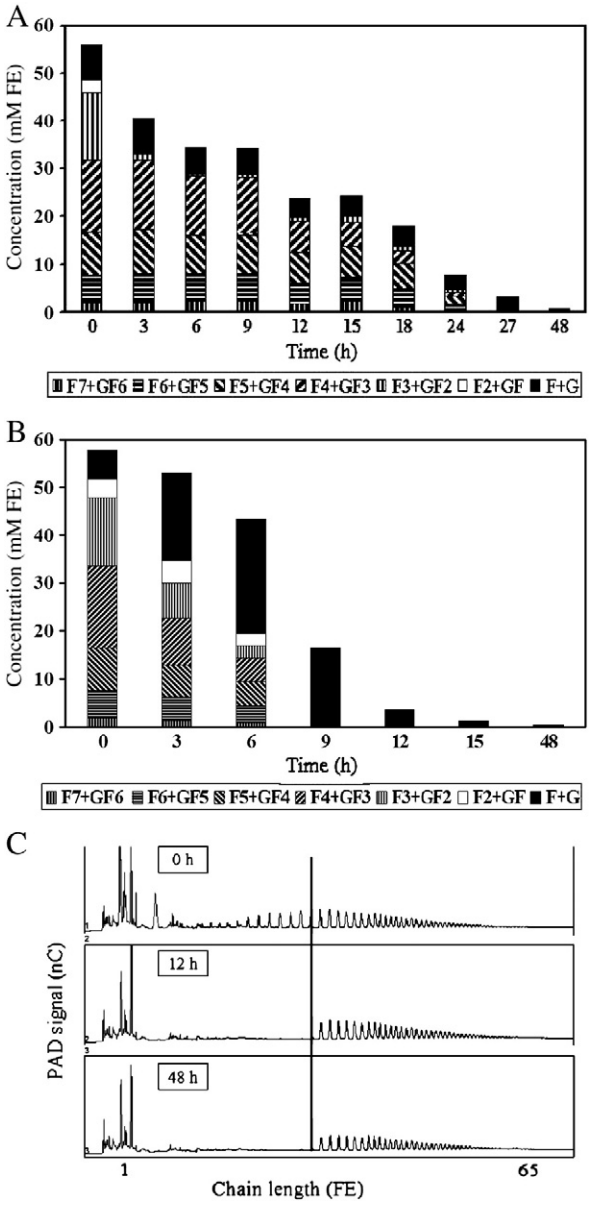

Fig. 2. Preferential breakdown of oligofructose by *Bifidobacterium adolescentis* LMG 10734 (A); non-preferential breakdown of oligofructose by *Bifidobacterium longum* LMG 11570 (B); and partial breakdown of inulin by *Bifidobacterium longum* LMG 11570 (C). The limit chain length for inulin degradation is indicated by a vertical line.