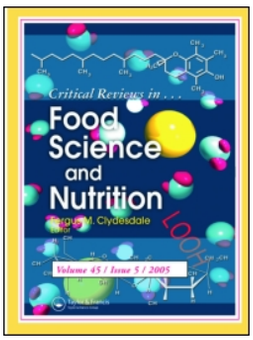

Critical Reviews in Food Science and Nutrition

ISSN: 1040-8398 (Print) 1549-7852 (Online) Journal homepage: http://www.tandfonline.com/loi/bfsn20

Exopolysaccharides Produced by Lactic Acid Bacteria and Bifidobacteria as Fermentable Substrates by the Intestinal Microbiota

Nuria Salazar, Miguel Gueimonde, Clara G. de los Reyes-Gavilán & Patricia Ruas-Madiedo

To cite this article: Nuria Salazar, Miguel Gueimonde, Clara G. de los Reyes-Gavilán & Patricia Ruas-Madiedo (2016) Exopolysaccharides Produced by Lactic Acid Bacteria and Bifidobacteria as Fermentable Substrates by the Intestinal Microbiota, Critical Reviews in Food Science and Nutrition, 56:9, 1440-1453, DOI: 10.1080/10408398.2013.770728

To link to this article: https://doi.org/10.1080/10408398.2013.770728

Accepted author version posted online: 12 Feb 2015.
Published online: 12 Feb 2015.

Critical Reviews in Food Science and Nutrition, 56:1440–1453, (2016)

ISSN: 1040-8398 / 1549-7852 online
DOI: 10.1080/10408398.2013.770728

Taylor & Francis
Taylor & Francis Group

Exopolysaccharides Produced by Lactic Acid Bacteria and Bifidobacteria as Fermentable Substrates by the Intestinal Microbiota

NURIA SALAZAR, MIGUEL GUEIMONDE, CLARA G. DE LOS REYES-GAVILÁN, and PATRICIA RUAS-MADIEDO

Department of Microbiology and Biochemistry of Dairy Products, Instituto de Productos Lácteos de Asturias – Consejo Superior de Investigaciones Científicas (IPLA-CSIC), Villaviciosa, Asturias, Spain

The functional food market, including products formulated to maintain a "healthy" gut microbiota, i.e. probiotics and prebiotics, has increased enormously since the end of the last century. In order to favor the competitiveness of this sector, as well as to increase our knowledge of the mechanisms of action upon human health, new probiotic strains and prebiotic substrates are being studied. This review discusses the use of exopolysaccharides (EPS), both homopolysaccharides (HoPS) and heteropolysaccharides (HePS), synthesized by lactic acid bacteria and bifidobacteria as potential prebiotics. These extracellular carbohydrate polymers synthesized by some gut inhabitants seem to be resistant to gastrointestinal digestion; these are susceptible as well to biodegradability by the intestinal microbiota depending on both the physicochemical characteristics of EPS and the pool of glycolytic enzymes harbored by microbiota. Therefore, although the chemical composition of these HoPS and HePS is different, both can be fermentable substrates by intestinal inhabitants and good candidates as prebiotic substrates. However, there are limitations for their use as additives in the food industry due to, on the one hand, their low production yield and, on the other hand, a lack of clinical studies demonstrating the functionality of these biopolymers.

Keywords Prebiotic, probiotic, functional food, health, EPS, gut

INTRODUCTION

For a long time, the production and preservation of foods was due to spontaneous fermentations carried out, among others, by the lactic acid bacteria (LAB) naturally present in the raw material. The development of food processing technologies led to the use of starter cultures allowing the control of the acidification process as well as the development of flavors and aromas typical of each product. Recently, the concept of "functional starter" has been introduced, referring to cultures that possess properties which contribute to food "microbial safety or offer one or more organoleptic, technological, nutritional, or health advantages" (Leroy and De Vuyst, 2004). Indeed, the market for foods that promote health and well-being, collectively known as "functional foods," has increased since the last decade of the previous century. In this context, foods containing probiotics constitute one of the oldest segments in the functional food market, and the dairy sector was pioneer in introducing these products. Probiotics have been defined as "live microorganisms which when administered in adequate amounts confer a health benefit on the host" (FAO/WHO, 2006). Most common probiotics used for human nutrition belong to the genera *Lactobacillus*, member of LAB group, and *Bifidobacterium* (Margolles et al., 2009). Nowadays, the new direction in this area is toward the development of target-specific foods, including probiotic strains rationally selected for a specific health benefit and designed for certain human populations (Arboleya et al., 2012).

Many bacteria, with either Gram-positive or Gram-negative envelopes, are surrounded by carbohydrate polymers named as

Address correspondence to Dr. Patricia Ruas-Madiedo, Department of Microbiology and Biochemistry of Dairy Products, Instituto de Productos Lácteos de Asturias – Consejo Superior de Investigaciones Científicas (IPLA-CSIC), Paseo Río Linares s/n, 33300 Villaviciosa, Asturias, Spain. E-mail: ruas-madiedo@ipla.csic.es

Color versions of one or more of the figures in the article can be found online at www.tandfonline.com/bfsn

exopolysaccharides (EPS). LAB and bifidobacteria are not exceptions and several strains have been described as EPS producers (recent review in Ruas-Madiedo et al., 2009a, 2009b, 2012). From an ecological point of view, it seems that these polymers do not act as carbon/energy reservoirs but are involved in the protection against harsh conditions and in the colonization of natural habitats of the EPS-producing bacteria (Gänzle and Schwab, 2009). Indeed some of these polymers, also known as capsular polysaccharides (CPS) when they are covalently attached to the cell surface forming a capsule, have been related with the virulence of certain pathogens (Cescutti, 2009). However, the involvement of EPS in the bacterial persistence and colonization of a given niche could also be of special relevance in the probiotic field. These polymers have been related to the interaction of probiotics with the gut ecosystem (Ruas-Madiedo et al., 2009b), although the exclusive implication of EPS, whether in combination with other extracellular bacterial factors, as well as the mechanism of such interaction, still remains to be elucidated. In addition, EPS-producing LAB have been used traditionally in dairy manufacturing due to the capability of EPS to confer viscosity and texture to fermented products (Hassan, 2008; Behare et al., 2009). Thus, the application of EPS-producing bacteria with probiotic traits could have a promising future in the functional food area. In this regard, this review focuses on the current available information about the implication of the EPS synthesized by LAB and bifidobacteria in the modulation of intestinal microbiota, that is, their feasibility to be used as fermentable substrates or prebiotics by the microbial community inhabiting our gut. In this context, after introducing the key microbiota player, the critical discussion of this review is divided in two sections corresponding to the main types of EPS synthesized by these Gram-positive bacteria: homopolysaccharides (HoPS), and heteropolysaccharides (HePS).

GUT MICROBIOTA

Composition and Activity

The human gastrointestinal tract (GIT) harbors a complex microbial ecosystem comprising 10^{14} microorganisms from hundreds of different species, including both beneficial and undesirable bacteria. This microbial community constitutes the so-called intestinal microbiota, and the pool of genes harbored by it is known as microbiome (Bäckhed et al., 2005). The number and distribution of these microorganisms varies along the gut, the stomach, and the upper bowel being sparsely colonized, while the colon is heavily populated (10^{11}–10^{12} bacteria/g contents, Figure 1). In addition to these differences along the gut, the development of GIT microbiota is a stepwise process depending on age (Figure 1). In early infancy, the microbiota is relatively simple, becoming more complex after weaning and reaching the adult state soon after (Salminen and Gueimonde, 2005; Koenig et al., 2011). Later on, senescence is related to further changes in the GIT microbiota (Mueller et al., 2006; Biagi et al. 2010). In addition to aging, the microbiota composition can be greatly influenced by several factors, including the specific genetic and microbial background of each individual, the diet, or the use of medication, among others (De Filippo et al., 2010; Dethlefsen and Relman, 2011). In spite of this complexity, during recent years the study of the GIT microbiota has attracted the attention of researchers mainly due to the important role it plays in the maintenance of host health (Round and Mazmanian, 2009; Sekirov et al., 2010; Tremaroli and Bäckhed, 2012).

Until recent years, our knowledge of gut microbiota composition and activity has mainly been based on the use of culture-dependent methods. The development of molecular culture-independent techniques has increased enormously our

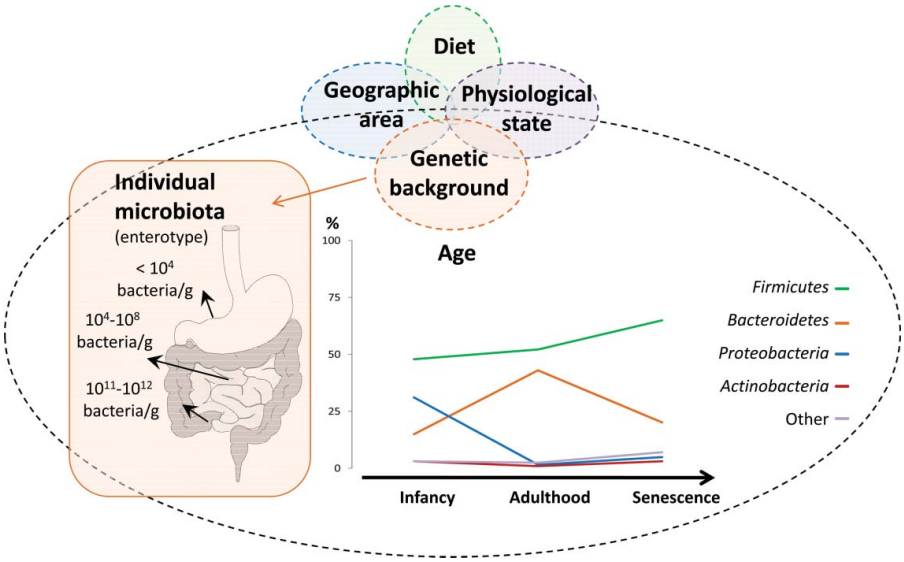

Figure 1 Intra- and inter-individual factors affecting the number and composition of human intestinal microbiota. GIT: gastrointestinal tract.

understanding of this microbial ecosystem and has demonstrated that only a minority of the members of the microbiota are cultivable; therefore, most of the intestinal microbes have not been studied yet. In addition, most of our microbiota data have focused on the fecal composition, which may not accurately represent the microbiota of the gut mucosa and proximal parts of the intestine. Nowadays microbiota research takes advantage of high-throughput techniques (*-omics*) for defining its composition in the gut and for sequencing the genomes of intestinal microorganisms. The development of massive DNA-sequencing methods has allowed extensive metagenomic studies to determine the human gut microbiome (Qin et al., 2010). These techniques have enormously enriched our knowledge on microbiome, allowing the establishment of different human enterotypes (Arumugam et al., 2011). The GIT microbiota of healthy adults includes nine bacterial divisions: *Firmicutes, Bacteroidetes, Proteobacteria*, A*ctinobacteria, Fusobacteria, Verrucomicrobia, Cyanobacteria, TM7*, and *Spirochaetes* (Vrieze et al., 2010). *Bacteroidetes* and *Firmicutes* represent between 80% and 90% of the population, whereas *Actinobacteria, Proteobacteria*, and *Fusobacteria* are subdominant groups with relative abundances that together do not exceed 14% of the population (Figure 1). Archaea, in turn, are mainly represented, up to date, by the microorganism *Methanobrevibacter smithii*. On the other hand, at division level the human intestine has a low bacterial diversity, with more than 15,000 different phylotypes being described at the species level, from which up to 70% are specific for each individual (Turnbaugh and Gordon, 2009). In addition, genomic analyses of intestinal microorganisms have provided data on their mechanisms of adaptation to the GIT environment and their metabolic capabilities. Such studies have allowed an understanding of the specialization of certain intestinal microbes in the gut and their ability to use different carbohydrates present in the intestine, and revealed mechanisms whereby these microorganisms interact with the host (Barrangou et al., 2003; O'Connell Motherway et al., 2011; Schell et al., 2002; Sela et al., 2008; Sonnenburg et al., 2005; Turroni et al., 2010).

As a whole, the intestinal microbiota displays an enormous metabolic versatility (Qin et al., 2010), allowing the use of different dietary and intestinal substrates, and leading to the production of metabolic products which may be beneficial to the host. These substrates include mucins, non-digestible oligosaccharides, and dietary fiber, among others. In the colon, these carbohydrates are fermented leading to the production of organic acids, short chain fatty acids (SCFA), and other fermentation products such as gases and ethanol. Unbranched SCFA, mainly butyric, propionic, and acetic acid, have been reported to be important for host health (Fukuda et al., 2011; Gao et al., 2009; Peng et al., 2009).

In spite of its importance to health, the "normal healthy" intestinal microbiota cannot be easily defined in microbiological terms, but it can be asserted that it is the microbial community that assists the host to maintain a healthy status (Peso-Echarri et al., 2011). As stated above, this microbiota plays an important role in human health not only due to its participation in the digestion process but also by its contribution to the gut barrier function (Ashida et al., 2012), gut development (Falk et al., 1998), and immune system maturation and modulation (Round and Mazmanian, 2009). Indeed, specific aberrancies on the gut microbiota composition have been reported in different intestinal and extra-intestinal diseases (DuPont and DuPont, 2011; Sekirov et al., 2010). The microorganisms present in our GIT have thus a significant influence on our health, providing the rationale for the development of nutritional strategies targeted to modulate the gut microbiota. Among these strategies, the use of prebiotic substrates has constituted a commonly used approach.

The Prebiotic Concept

The prebiotic term was defined for the first time as "a non-digestible food ingredient that beneficially affects the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon, and thus improves host health" (Gibson and Roberfroid, 1995). This definition was later revised by extending the site of action to other areas of the GIT, as follows: "A selectively fermented ingredient that allows specific changes, both in the composition and/or activity in the gastrointestinal microflora that confer benefits upon host well-being and health" (Gibson et al., 2004). Later on, the International Association of Probiotics and Prebiotics (ISAPP) slightly modified the definition by indicating that "a dietary prebiotic is a selectively fermented ingredient that results in specific changes in the composition and/or activity of the gastrointestinal microbiota, thus conferring benefit(s) upon host health" (ISAPP, 2008). The gut microbiota is now perceived as a key player for host health and well-being, and a composition in which potentially beneficial microorganisms and their metabolic activities (mainly saccharolytic genera/species) are enriched to the detriment of the potentially harmful ones (mainly the proteolytic–putrefactive genera/species) is considered optimal (Cummings et al., 2004). Traditionally *Lactobacillus* and *Bifidobacterium* have been perceived as the microbial targets of prebiotic action. However, given the complexity of interactions occurring in the intestinal ecosystem among the different populations, the recent advances in the understanding of the microbial physiology, and the key role that diverse microorganisms play in such environment, it is likely that the prebiotic concept may also be expanded toward other intestinal microbial genera, such as, for example, *Eubacterium, Faecalibacterium*, and *Roseburia* (FAO/WHO, 2007; Roberfroid et al., 2010). Recently, two expert panels from the International Life Sciences Institute (ILSI) have published a detailed revision of the prebiotic concept and their effects on metabolism and health benefits, which cover, in addition to the modulation of gut microbiota, the mineral absorption and bone health, the intestinal and immune functions, the intestinal barrier function and risk of infection, and tentatively, colon cancer, obesity, and related disorders (Roberfroid et al., 2010).

In order to be considered as prebiotic, a food ingredient must meet the following requirements: (i) be resistant to gastric and intestinal digestion, (ii) not be absorbed in the small intestine, (iii) be fermented by the intestinal microbiota, and (iv) produce a beneficial effect on host health through the selective stimulation of microbial populations or their metabolic activities. The diet-derived non-digestible carbohydrates provide the major energy source for the microbiota in the colon, and the amount and type of prebiotic substrates have a deep impact in the composition and activity of this microbial community (Flint, 2012). Table 1 shows some of the currently studied non-digestible oligosaccharide (NDO) substrates, although, for historical reasons, the largest amount of scientific evidence of the prebiotic effect has been obtained so far for inulin-type fructans, fructo-oligosaccharides (FOS), galacto-oligosaccharides (GOS), and lactulose (Charalampopoulos and Rastall, 2012). Other compounds that are currently being considered as prebiotics, although with less scientific evidence, mostly include complex carbohydrates from plants or those that are industrially synthesized from milk and vegetable components using enzymatic and chemical procedures such as lactosucrose, xylo-oligosaccharides (XOS), isomalto-oligosaccharides (IMO), soybean oligosaccharides (SBOS), and gluco-oligosaccharides (Casci and Rastall, 2006; Crittenden and Playne, 2009). There is increasing scientific evidence of the potential prebiotic effect of resistant starch, arabinoxylan, and vegetable gums, although few studies are available (Bird et al., 2010; Broekaert et al., 2011; Candela et al., 2010). In addition, it is well known that the human milk oligosaccharides (HMO) are used as carbon and energy sources by the intestinal microbiota of breastfed infants, also having a bifidogenic (increasing levels of *Bifidobacterium*) effect (Sela and Mills, 2010; Xiao et al., 2010).

As previously indicated, fermentation of prebiotic substrates in the gut mainly leads to the production of SCFA. In general, substrates that are quickly fermented give rise to the production of larger amounts of lactic and acetic acids; whereas, those fermented slowly lead to the production of more butyric acid as the final product of fermentation by the intestinal microbiota (Nuggent, 2005; Rossi et al., 2005; Van de Wiele et al., 2007). Qualitative and quantitative changes in the microbial populations or in the substrates available in the intestinal ecosystem derived from the consumption of dietary NDO generate, in turn, changes in the production of end microbial metabolic products such as SCFA, which are produced by many saccharolytic bacteria in the colon. Some intermediate compounds of these fermentations include, among others, ethanol and organic acids (lactic, succinic, and pyruvic). These, together with some partially degraded carbohydrate polymers, may also be finally metabolized to SCFA by microbial *cross-feeding* and other complex interaction mechanisms in which specific intestinal microbial groups can be involved (Flint et al., 2008; Saulnier et al., 2009; De Vuyst and Leroy, 2011). Then the beneficial effects attributed to prebiotics result from the specific action in the human body of SCFA produced by microbial fermentation of these substrates, as well as from the ability of these compounds to modify the intestinal microbiota composition and/or activity; this results in subsequent modifications in the capacity of gut microbiota to interact with each other and with the eukaryotic cells of the host.

Considering all that is indicated above, it would not be surprising if other complex carbohydrates, not considered so far, also had the ability to interact at the intestinal level. The potential influence of these carbohydrates in microbiota composition and activity, as well as in host health, deserves further attention. In this regard, some of the most promising candidates are the EPS synthesized by LAB, bifidobacteria, and other beneficial microorganisms that can inhabit, or with foods reach, the human gut.

Models to Study the Prebiotic Potential of EPS

Several approaches have been used to determine the susceptibility of EPS polymers to human gastrointestinal digestion but

Table 1 Some examples of non-digestible oligosaccharides (NDO) with known or potential prebiotic effect (modified from Casci and Rastall, 2006; Crittenden and Playne, 2009)

Substrate (industrial process)	NDO types	Constituent monosaccharides	Some known natural dietary sources
Sucrose (transglycosylation)Inulin (hydrolysis)Plant (direct extraction)	Fructo-oligosaccharides (FOS): levan and inulin-type	$(Fru)_n$-Glc(β-2→6), or (β-2→1) linkages	Vegetables (beet, chicory, artichoke, onion, garlic, leek), grains (wheat)
Sucrose/Lactose(transglycosylation)	Lactosucrose	Gal-glc-fru	Chemical synthesis
Lactose (isomerization)	Lactulose	Gal-fruc	Chemical synthesis
Lactose (hydrogenation)	Lactitol	Gal-glucitol	Chemical synthesis
Lactose (transglycosylation)	Galacto-oligosaccharides (GOS)	$(Gal)_n$–Glc	Chemical synthesis
Starch (dextran hydrolysis and transglycoslylation)	Isomalto-oligosaccharides (IMO)	$(Glc)_n$ (α-1→6)	Fermented foods and sugars (sake), soybean, honey
Starch (direct extraction)	Resistant starch	—	Maize starch
Beet (direct extraction)	Raffinose	Gal-glc-fru	Vegetables (beans, cabbage, broccoli, asparagus) and grains
Beet (direct extraction)	Stachyose	$(Gal)_2$-glc-fru	Vegetables (beans, cabbage, broccoli, asparagus) and grains
Soybean (direct extraction)	Soybean oligosaccharides (SBOS)	$(Gal)_n$-glc-fru	Soybean
Corn cobs (arabinoxylan hydrolysis)	Xylo-oligosaccharides (XOS)	$(Xyl)_n$ (β-1→4)	Fruits, vegetables, milk, honey

Fruc: fructose, Glc: glucose, Gal: galactose; Xyl: xylose.

most of these follow a common diagram aiming to chemically in vitro simulate the conditions of the upper-gut transit. Typically, the polymers are submitted to discontinuous or sequential challenges, which are also often applied to the EPS-producing strains, such as gastric, duodenal, and intestinal solutions under variable conditions mimicking the human physiology (Figure 2A). Afterwards, for the quantification of the EPS hydrolysis, different methodologies have been used; these involve the measurement of monosaccharide released by, for example, the phenol-sulfuric method and by ion-exchange high-performance liquid chromatography (HPLC), or the quantification of the EPS degradation – i.e. changes in molecular weight (Mw) – by means of size exclusion chromatography (SEC) in HPLC systems, or using nuclear magnetic resonance (NMR).

In order to demonstrate the biodegradability of EPS, or any potential prebiotic, by the intestinal microbiota in vitro, gut fermentation systems are very useful tools for investigating the microbial processes of carbohydrate fermentation; these models represent an outstanding approach to perform studies frequently challenged in humans and animals due to economic, ethical, and social concerns. These in vitro models not only analyze the behavior of defined single or mixed cultures, most often lactobacilli and/or bifidobacteria, but also the dynamics of undefined microbiota currently obtained from fecal material. The methodology applied covers from the simplest batch-culture fermentations to the most sophisticated single or multiple pH-controlled, continuous-culture systems (Figure 2B); these multistage systems are designed to mimic environmental conditions that occur in different parts of the colon or even to simulate the whole human gut, thus including the stomach and the small intestine conditions (Marzorati et al., 2011; Sarbini et al., 2011; Tabernero et al., 2011; van den Abbeele et al., 2010, 2012). New models still under development are trying to include the eukaryotic (host) component, in most cases mucin and/or intestinal-epithelium cellular lines, aiming to take into the equation the putative interactions among host, microbiota, and prebiotics (Cencic and Langerholc, 2010; Yu et al., 2012). However, this is a challenging issue and it is hard to imagine that the ideal in vitro model could ever be designed to mimic the in vivo situation. Finally, a variety of culture-dependent and culture-independent techniques, including the most advanced *-omics*, are applied to study the qualitative and/or quantitative variations of the bacterial community, or their metabolic activity induced by the prebiotic substrates feeding the microbial system (Fraher et al., 2012; Hyötyläinen, 2012).

HOMOPOLYSACCHARIDES AS MODULATORS OF THE GUT MICROBIOTA

As its name suggests, HoPS comprise a single monosaccharide type, either glucose (glucans) or fructose (fructans)

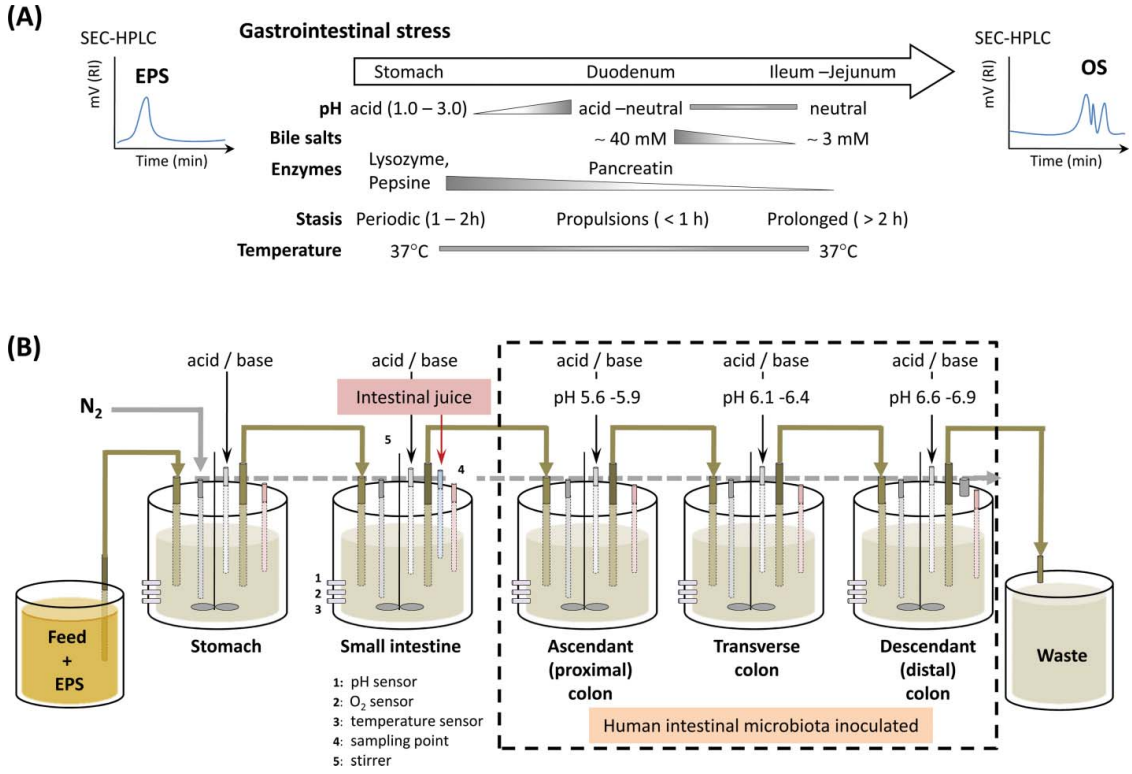

Figure 2 In vitro models used to study prebiotic potential of substrate candidates. (A) Gastrointestinal digestion challenges (modified from de los Reyes-Gavilán et al., 2011), and (B) bio-digestibility by the intestinal microbiota (modified from van den Abbeele et al., 2010). EPS: exopolysaccharides, OS: oligosaccharides, SEC: size exclusion chromatography.

(Monsan et al., 2001). Among LAB, a sub-classification has been established depending on the linkage type and the position of the carbon involved in the bond: α-glucans (dextran: α-D-Glc(1→4), mutan: α-D-Glc(1→3) and alternan: (α-D-Glc (1→6)/α-D-Glc(1→3), among others), β-glucans (β-D-Glc (1→3) with side chain-linked (1→2)), and β-fructans (levan-type: β-D-Fru(2→6) and inulin-type: β-D-Fru(2→1)); up to date HoPS have not been described nor purified in *Bifidobacterium* genus (Ruas-Madiedo et al., 2009a). Regarding α-glucan and β-fructan synthesis, a single type of extracellular enzyme belonging to the glycosyl hydrolase (GH) family is required; these are named glucansucrases (GH family 70) and fructansucrases (GH family 68) for glucans and fructans biosynthesis respectively (http://www.cazy.org). These enzymes are able to catalyze the polymerization of HoPS using sucrose as a substrate donor of the corresponding monosaccharide (Korakli et al., 2006; van Hijum et al., 2006). On the other hand, the production of β-glucan has been mainly described in LAB isolated from alcoholic-fermented beverages, and its synthesis involves a glucosyltransferase, a membrane-associated enzyme whose mechanism of action is still poorly understood but it does not need sucrose as substrate (Werning et al., 2006).

The yield of HoPS produced by LAB is low compared with other bacterial EPS used in the food industry. In general, the HoPS production level described in LAB is higher than 1 g/L, with a few strains reaching values of up to 10 g/L, as in the case of *Lactobacillus reuteri*, Lb121 is able to simultaneously synthesize α-glucan and β-fructans (van Geel-Schutten et al., 1999). The size of these homopolymers, which can be measured in terms of molecular weight, is commonly higher than 10^3 kDa and varies according to the producing strain (Ruas-Madiedo et al., 2009a, 2009b). The relatively simplest chemical composition of these HoPS could be the key parameter determining their potential to act as fermentable substrates by the intestinal microbiota. Indeed, some of these HoPS (β-glucans) have a similar chemical composition and linkage types to that of the most studied prebiotics, such as inulin and FOS. The most remarkable difference among these molecules is the size, which is directly related to their degree of polymerization (DP); FOS and inulin are short oligosaccharides (DP < 30, Mw ~ 5 kDa), whereas HoPS are branched polymers of high molecular weight (1000 kDa). Nevertheless, in order to demonstrate the prebiotic potential of these bacterial polymers, these must fulfill the criteria mentioned previously.

Tolerance of HoPS to Gastrointestinal Digestion

There is a common assumption that prebiotic substrates should not be digested nor absorbed in the upper part of the GIT (Roberfroid et al., 2010). This statement has been inferred from the analysis of diet component that arrived undigested to the colon; however, there is a notorious lack of standardized procedure to demonstrate the non-digestibility of any prebiotic candidate under the upper GIT conditions. Similarly, no data are available regarding the in vitro or in vivo gastrointestinal digestion of HoPS from LAB origin. In a recent article, Hongpattarakere and co-workers (2012) have detected EPS-producing LAB in the gut of marine animals; given that these polymers were detected in culture medium supplemented with sucrose, they probably are HoPS type (data not indicated by the authors). These authors have detected that the polymers tested remained almost undigested under GIT challenges. Therefore, although there is no scientific proof for HoPS tolerance to gastrointestinal transit, the big size of these polymers (which will make difficult their acid and enzymatic hydrolysis), together with its chemical composition (resembling that of prebiotic inulin and FOS substrates), would allow us to hypothesize that LAB-HoPS could reach the colon almost undigested, where these could be used as fermentable substrates. Nevertheless, this hypothesis deserves further demonstration.

Biodegradability of HoPS by the Gut Microbiota

Several studies have assessed the biodegradability by fecal microbiota of different EPS, either HoPS or HePS types, synthesized by LAB and bifidobacteria (Table 2). Results obtained with HoPS, which have the simplest primary structure, showed that most of these were susceptible fermentation. The β-fructan (levan-type) EPS isolated from *Lactobacillus sanfranciscensis*, a bacteria commonly employed in sourdough fermentation, was able to modify the composition of gut microbiota in fecal fermentations (Dal Bello et al., 2001). The results from the fingerprinting technique used (polymerase chain reaction (PCR)–temperature gradient gel electrophoresis (DGGE)) showed that the batch cultures supplemented with HoPS had a bifidogenic effect, in the same way as inulin, and also increased the occurrence of other gut bacterial species such as *Eubacterium biforme*. Interestingly, these authors did not find an increase in the *Bifidobacterium* population when commercial levan (from *Erwinia herbicola*) was used, thus suggesting the occurrence of structural differences between both bacterial levan-type polymers, which, in turn, conferred differential susceptibilities on microbial degradability. The growth and metabolic activity of different bifidobacteria species in the presence of the levan-type HoPS from *Lb. sanfranciscencis* was further confirmed in single-species, pH-controlled fermentations (Korakli et al., 2002). Probably, specific members of the microbiota are able to breakdown the high molecular weight HoPS rendering shorter (low degree of polymerization) oligosaccharides, which could be directly used by other intestinal bacteria. In this way, it has been shown that branched gluco-oligosaccharides (ranging from DP 2 to 7) synthesized by *Leuconostoc mesenteroides*, using the same enzymes involved in α-glucan synthesis, are fermented by bacteria considered as probiotics (Chung and Day, 2002). In addition, indirect evidence supporting the use of HoPS (β-glucan) as a fermentable source by the intestinal microbiota has been obtained as a secondary outcome by Märtensson and co-workers (2005) in a study with adult human

Table 2 Evidences of biodegradability by intestinal microbiota of EPS synthesized by different LAB and bifidobacteria

EPS-type	Model – microbiota	EPS-producing bacteria	Tested	EPS biodegradability	Reference
HoPS					
β-fructan	Batch, pH-free cultures – human feces	*Lb. sanfranciscensis* LTH1729 and LTH2590	Polymer	Yes	Dal Bello et al., 2001
β-fructan	Single species, pH-controlled cultures – bifidobacteria	*Lb. sanfranciscensis* TMW1.392	Polymer	Yes	Korakli et al., 2002
β-glucan	Human trial (oat-fermented product)	*Pediococcus damnosus* 2.6	Strain	Yes	Märtensson et al., 2005
Unknown	Continuous, pH-controlled three-stage system – human feces	*W. cibaria* A2	Polymer	Yes	Hongpattarakere et al., 2012
HePS					
	Batch, pH-free cultures – human feces	*Lc. lactis* subsp. *cremoris* B40*Lb. helveticus* Lh59*Lb. sakei* 0–1*St. thermophilus* Sfi12*St. thermophilus* Sfi20*St. thermophilus* Sfi39	Polymer Polymer Polymer Polymer Polymer Polymer	No No No Yes No Yes	Ruijssenaars et al., 2000
	Continuous, pH-controlled, three-stage system – infant feces	*Lb. rhamnosus* RW-9595M	Polymer	No	Cinquin et al., 2006
	Batch, pH-free cultures – human feces	*Bifidobacterium* ssp. (11 strains)	Polymer	Yes	Salazar et al., 2008
	Batch, pH-controlled cultures – human feces	*B. longum* IPLA E44*B. animalis* subsp. *lactis* IPLA R1	Strain Strain	Yes Yes	Salazar et al., 2009
	Rat model (bifidobacterial milk suspensions)	*B. longum* IPLA E44*B. animalis* subsp. *lactis* IPLA R1	Strain Strain	Yes Yes	Salazar et al., 2011

volunteers. Three intervention groups were compared: one was receiving a fermented oat-based product and the second the same product but co-fermented with the β-glucan-producing strain *Pediococcus damnosus* 2.6, which conferred a "ropy" viscosity on the product. The third group (placebo) was receiving a dairy-based fermented product. A significant increase in total bacteria and bifidobacteria counts was observed in fecal samples of the volunteers who received the product containing the β-glucan-producing bacteria only when compared with the placebo group. However, it is not clear whether the capability to modulate the composition of the human intestinal microbiota was exclusively due to the β-glucans from bacteria present in the "ropy" oat-based product, since the oat itself is also a source of plant-origin β-glucans.

These results seem to indicate that HoPS could be used as fermentable substrates by the intestinal microbiota due to their, relatively, simple primary structure. However, the presence of glycosyl hydrolases (glucosyl- and fructosyl-hydrolase types) able to breakdown the side monosaccharides of the homopolymers has not been detected yet in the gut ecosystem. Besides, the lack of in vivo evidences showing the efficacy of HoPS as prebiotic substrates is remarkable.

HETEROPOLYSACCHARIDES AS MODULATORS OF THE GUT MICROBIOTA

The HePS are complex polymers built on repeating units comprising different monosaccharides, mainly glucose, galactose, and rhamnose, and, to a lesser extent, N-acetylated monosaccharides (N-acetyl-glucosamine and N-acetyl-galactosamine); other monosaccharides can be found in some specific HePS as well as organic and inorganic (glucoronic acid, acetyl groups, glycerol, phosphate, etc.) substituents. Monomers of the repeating unit backbone are linked by different types of osidic bonds, and side groups can be present as well; this results in a wide variety of HePS with specific physical properties and water solubility, which, thereby, confer different technological and biological capabilities. More than 45 different repeating units comprising combinations from two to eight monomers have been described up to date by means of NMR techniques (De Vuyst et al., 2001; Broadbent et al., 2003; Ruas-Madiedo et al., 2009a), but only a few corresponding to EPS are synthesized by bifidobacteria (Ruas-Madiedo et al., 2012). In accordance with this chemical diversity, the enzymatic machinery involved in the synthesis of HePS is much more complex than that of HoPS. In LAB, the genes encoding these enzymes are organized in *eps* clusters, showing a highly functional organization and an operon-like structure (Jolly and Stingele, 2001; Broadbent et al., 2003; Ruas-Madiedo et al., 2009a). Often LAB–*eps* clusters harbor genes encoding proteins predicted to be involved in regulation, glycosyltransferases for the synthesis of the repeating units, as well as proteins for chain length determination, polymerization, and export. On the contrary, the *eps* clusters described in the *Bifidobacterium* genomes do not show this conserved structural organization and a high inter- and intra-species variability has been denoted (Ruas-Madiedo et al., 2012).

The HePS production level described in LAB and bifidobacteria is lower than that of HoPS. Production is strain-dependent

and can be modified by the composition of culture media, pH value, temperature, incubation time, etc. (Deggest et al., 2001). The reported yield is also highly dependent on the method used for biomass collection as well as for HePS purification and quantification; therefore, the lack of standardized methods to determine HePS production makes difficult to give an accurate yield. The values reported in literature oscillates from 25 to 600 mg/L (Ruas-Madiedo et al., 2009b) and only few strains are able to produce higher amounts under optimized growth conditions; being the case, for example, of *Lactobacillus rhamnosus* RW-9595M rending 2 g of polymer per liter of culture (Bergmaier et al., 2005). The data about HePS yield in bifidobacteria are very scarce, but are in the same range as that reported for LAB polymers, and are submitted to the same methodological dependence (Ruas-Madiedo et al., 2012). Regarding the molecular weight of HePS, it has been described as a broad range, varying from 10^4 to 10^6 Da, and the simultaneous occurrence of more than one chromatographic peak of a different size in the polymer fraction synthesized by a given strain is somewhat frequent in both LAB- and bifido-HePS (Mozzi et al., 2006; Salazar et al., 2009a; Ruas-Madiedo et al., 2009a). This parameter is of special relevance for the technological functionality of HePS; that is, the ability of the polymer to confer viscosity and texture to fermented milk depends, among other factors, on its size. Besides, the molecular weight of HePS could also be a key parameter related with the capability to interact with the host (Hidalgo-Cantabrana et al., 2012). For instance, it seems that lactobacilli and bifidobacteria producing HePS of big size induce an immune suppressor profile in cultures of human immune cells and in animal models (Fanning et al., 2012; López et al., 2012; Nikolic et al., 2012). In addition, these polymers have been directly related to the capability of the producing strain to survive the challenges of innate immune barrier at the intestinal mucosa (Lebeer et al., 2010). Therefore, the HePS synthesized by LAB and bifidobacteria inhabiting the gut could have pivotal relevance for the maintenance of the healthy status of this ecosystem, and thereby that of the host. However, it is worth to point out that more studies are required to demonstrate that these effects are unequivocally or exclusively due to HePS, and not to a combination with other surface components (e.g. proteins, pilus-like appendages, teichoic or lipoteichoic acids, etc), which can be present in the purified HePS fraction or are part of the HePS-producing bacterial envelope.

Tolerance of HePS to Gastrointestinal Digestion

The experimental procedure indicated previously (Figure 2A) has been used to determine the gastrointestinal hydrolysis of a few HePS synthesized by LAB isolated from different ecosystems such as foods and animal guts. This is the case of heteropolymers from *Lactococcus lactis* subsp. *cremoris* (Looijesteijn et al., 2001), *Streptococcus thermophilus* and *Lactobacillus casei* (Mozzi et al., 2009), or *Lactobacillus paraplantarum* (Nikolic et al., 2012), and human origin species of *Bifidobacterium* (Salazar et al., 2009a; Leivers et al., 2011). Results obtained from most of these studies show a common trait: bacterial HePS, independent of the producing strain and origin, exhibit high resistance to gastrointestinal digestion. Thus, it seems that these bacterial polymers could fulfill one of the requisites to be considered as prebiotics. However, there is a lack of in vivo evidence directly showing the undigestibility of bacterial HePS in the upper GIT. This could be due, on one hand, to a methodological limitation for the isolation of these polymers from the in vivo ecological niches and, on the other hand, to the scarce yield of bacterial HePS that limits the amount of substrate that can be used to feed animal (or human) models. Nevertheless, there is indirect in vivo evidence showing that some of these biopolymers are resistant to gastrointestinal stress and these could exert a protective function on HePS-producing bacteria in the gut ecological niche (Lebeer et al., 2010; Salazar et al., 2011; Fanning et al., 2012).

Biodegradability of HePS by the Gut Microbiota

One of the earliest studies analyzing the biodegradability of HePS was performed by Ruijssenaars and co-workers (2000), who followed, in cultures of homogenized feces (fecal slurries), the concentration of sugars released from the purified LAB-HePS present as supplement in the culture medium (Table 2). These LAB were from food origin and four out of the six biopolymers were not fermented by the human gastrointestinal microbiota, those synthesized were *Lc. lactis* ssp. *cremoris* B40, *Lactobacillus sakei* 0–1, *St. thermophilus* Sfi20, and *Lactobacillus helveticus* Lh59. Meanwhile, the EPS from *St. thermophilus* Sfi39 and Sfi12 were degraded to higher degree, probably due to the simple structures of the repeating units of these polymers, which presented a side β-galactose residue that could be easily released by the enzymatic machinery of microbiota. This was one of the first studies correlating the physicochemical composition of HePS with their susceptibility to biodegradation and, therefore, with their potential biological properties in the gut ecosystem. Similarly, the structural complexity of the HePS synthesized by *Lb. rhamnosus* RW-9595M, comprising a heptasaccharide repeating unit (van Calsteren et al., 2002), could explain its incapability to be metabolized by infant microbiota (Cinquin et al., 2006). This result was independent of the culture conditions in the continuous, three-stage colonic system used as a model: different inocula of immobilized infant feces and/or different nutrient supplements added to the carbohydrate-free basal medium were tested, following in-parallel FOS fermentations as a positive control (Cinquin et al., 2006).

The studies previously gathered were carried out with polymers purified from the strains of food origin; besides, it has also demonstrated the occurrence of HePS-producing strains in the gut of animals, although the intriguing question that remains to be elucidated is whether EPS are also synthesized by inhabitants of the intestinal microbiota under physiological (in vivo)

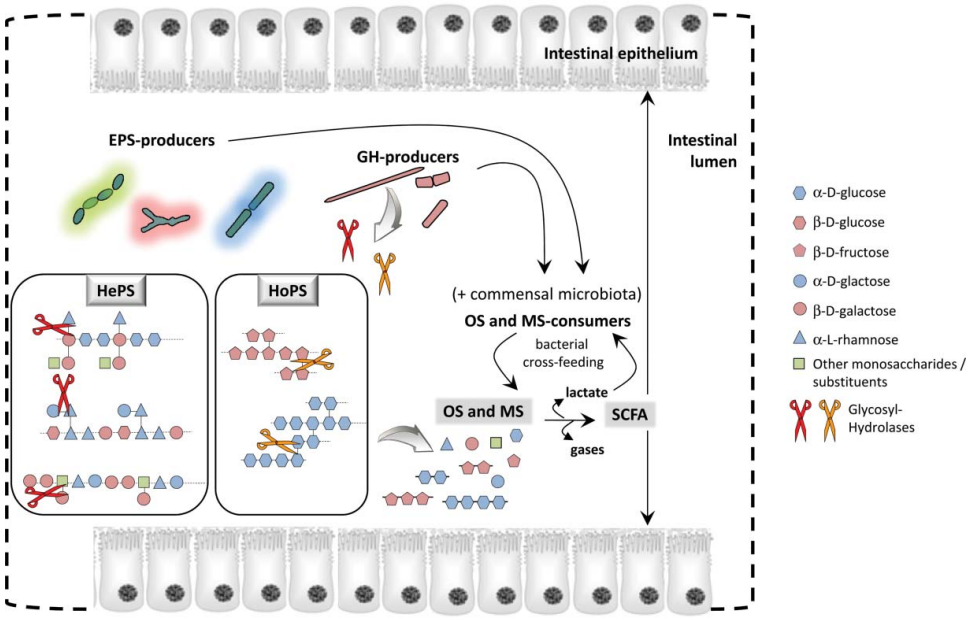

Figure 3 Schematic representation of the microbial cross-interactions established between the EPS-producers, the EPS-degraders and the OS–MS consumers in the gut environment. HePS: heteropolysaccharides, HoPS: homopolyaccharides, OS: oligosaccharides, MS: monosaccharides, and SCFA: short chain fatty acids.

conditions. Our group has published the first genetic and phenotypic screening of a collection of human fecal strains for the detection of EPS-producing lactobacilli and bifidobacteria. From 362 human isolates, 60 strains (35 bifidobacteria and 25 lactobacilli) presented an EPS-producing mucoid or ropy phenotype, and the presence of *eps* genes was detected in 35% of them, only those genes involved in HePS synthesis being positive (Ruas-Madiedo et al., 2007). Later on, the physicochemical characterization of 21 of these polymers (11 from *Bifidobacterium* and 10 from *Lactobacillus*) confirmed their heteropolymer-like nature (Salazar et al., 2009a). In a step forward, the capability of the HePS synthesized by these gut inhabitants to be used as a fermentable carbon source by the intestinal microbiota was explored. Therefore, 11 polymers (bifido-HePS) purified from human-origin strains belonging to the species *Bifidobacterium animalis, Bifidobacterium longum*, and

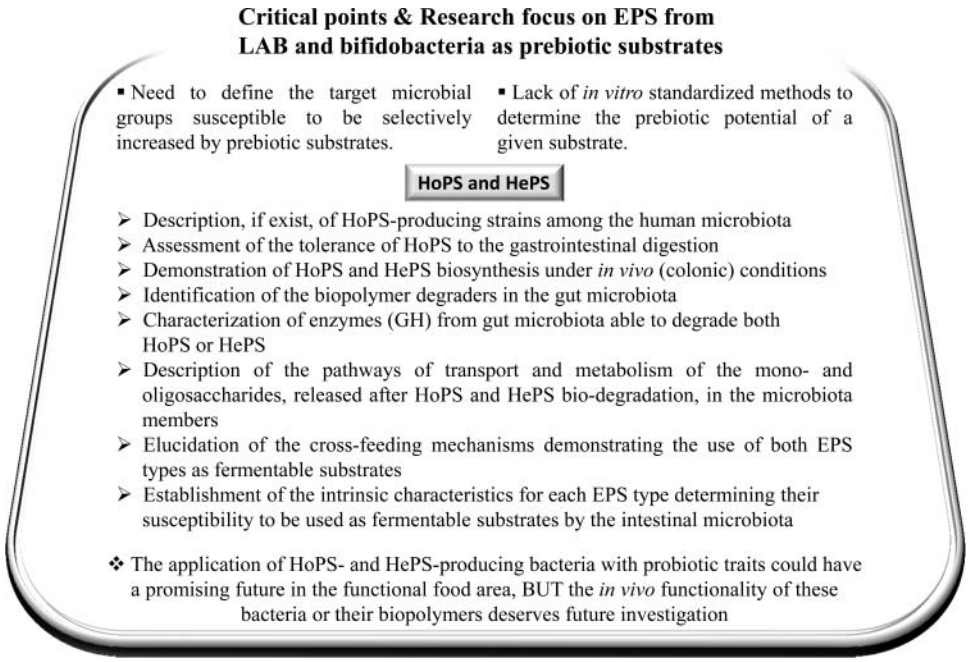

Figure 4 Critical points and research focus box about the EPS from LAB and bifidobacteria as prebiotic substrates.

Bifidobacterium pseudocatenulatum were chosen as the model of study. Initially, non-pH-controlled batch cultures of human fecal slurries obtained from three healthy donors (cultured separately) were performed (Salazar et al., 2008). Results obtained showed high inter-individual variations, but the quantitative PCR data revealed a tendency toward the enrichment of the genus *Bifidobacterium* promoted by bifido-HePS in a similar way as that of the prebiotic inulin. Besides, the PCR–DGGE banding patterns showed a qualitative rearrangement in other gastrointestinal microorganisms, and the SCFA profile obtained suggested a shift in the production of microbial metabolites. Remarkably, a decrease in the ratio of acetic acid to propionic acid, proposed by some authors as a hypolipidemic indicator (Delzene and Kok, 2001), was observed. The values of this ratio obtained for the HePS-E44 (from *B. longum*) and C52 (from *B. pseudocatenulatum*) were even lower than that observed for inulin (Salazar et al., 2008). Therefore, these results showed that the human origin HePS were also able to modulate the composition and activity of colonic microbiota in a polymer-dependent way, thus strongly supporting the fact observed with the food origin HePS; that is, the physicochemical characteristics of biopolymers must account for their capability to act as fermentable substrates independent of the isolation niche of the HePS-producing strain. In order to provide new data reinforcing this hypothesis, two polymers of different origin were tested in another in vitro model: the HePS from the human gut isolate *B. longum* IPLA E44 and that from the dairy product isolate *B. animalis* IPLA R1 were analyzed in human fecal-slurry, pH-controlled batch cultures simulating the conditions in the distal part of the gut (Salazar et al., 2009b). The fluorescence in situ hybridization (FISH) pattern confirmed that both HePS promoted changes in different microbial populations, including a bifidogenic effect, as well as shifts in the production of SCFA and lactate.

As far as we know, only one in vivo (animal) study has analyzed the potential role of bifido-HePS as modulators of the intestinal microbiota (Table 2), although the effects detected cannot be definitively assigned to the polymers since the animals were fed with the whole HePS-producing bacteria. In this study, Wistar rats were used to test the safety and microbiota modulating capability of two HePS-producing strains: *B. longum* IPLA E44 and *B. animalis* subsp. *lactis* IPLA R1 (Salazar et al., 2011). Three groups of rats were fed with each bifidobacteria strain suspended in skimmed milk (at doses of 10^9 cfu/day) or with skimmed milk (placebo). Rats fed with both HePS-producing bifidobacteria presented higher *Bifidobacterium* counts than the placebo group; remarkably, independent of the strain orally administered, the species *B. animalis* was predominant in the caecum content and feces of both bifidobacteria-fed groups. Besides, rats receiving bifidobacteria had significantly higher total SCFA concentration in feces, but significantly lower in caecum content, than in the placebo group, thus suggesting a displacement in the production of SCFA to parts of the colon beyond the caecum induced by the EPS-producing bifidobacteria. The technique used to follow the dynamics of the intestinal microbiota (PCR–DGGE) showed that the HePS-producing strain *B. animalis* subsp. *lactis* IPLA R1 was able to significantly increase microbial diversity (increased number of bands) with respect to the rats fed with *B. longum* IPLA E44 or placebo. This result suggested that both HePS-producing strains could have different levels of resistance during gastrointestinal transit, *B. animalis* IPLA R1 being more robust. Indeed, in vitro evidence indicates that species *B. animalis* subsp. *lactis* better supports the harsh upper gastrointestinal conditions than *B. longum* (de los Reyes-Gavilán et al., 2011). The apparently poor in vivo survival of *B. longum* IPLA E44 could be related to an intrinsic characteristic of this species, but it also indicates that the HePS surrounding the producing bacteria seemed not to confer enough protection during gastrointestinal transit. Against this, in vivo studies carried out with different models of HePS-producing lactobacilli or bifidobacteria and their respective HePS-KO mutants suggest that certain HePS could create a protective micro-environment allowing upper gastrointestinal transit and favoring survival/persistence in intestinal niche (Denou et al., 2008; Lebeer et al., 2010; Fanning et al., 2012). However, the involvement of other bacterial surface components in this protective effect cannot be discarded.

CONCLUSIONS AND FUTURE RESEARCH

From this overview, a clear picture can be drawn about the capability of HoPS and HePS synthesized by LAB and bifidobacteria to act as fermentable substrates by the intestinal microbiota: both bacterial EPS types seem to be resistant to gastrointestinal digestion but differ in their susceptibility to be degraded by the gut microbiota. This last feature must be directly related, on the one hand, to the physicochemical properties of the biopolymers and, on the other hand, to the pool of hydrolytic enzymes harbored by gut microorganisms capable of breaking them down. In this way, the biodegradability of both EPS types in the colon must be greatly influenced by the gut microbiota composition and vice versa, the suitability of LAB- and bifido-EPS to act as fermentable substrates could promote rearrangements of different bacterial populations throughout cross-feeding mechanisms. Therefore, we could hypothesize that in a physiological (in vivo) situation, the EPS, either HoPS or HePS, synthesized by intestinal bacteria could act as carbon-source reservoirs for microbiota and these could be used throughout cross-interactions established among microbiota members (Figure 3). In both the cases, after EPS bio-digestion, the released short degree of polymerization oligosaccharides and monosaccharides could be used as fermentable substrates by several microbiota members, i.e. the EPS-producers, the EPS-degraders, or any the gut inhabitant, which will render SCFA and lactic acid, among other metabolites. The SCFA and lactate could be reused by microbiota members throughout cross-feeding mechanisms, and/or could act as a fuel for the intestinal epithelium. This scheme could be a successful microbial strategy to obtain nutrients (carbon source)

in the colon, an ecological niche characterized by extreme starving conditions and high microbial antagonism. The final beneficiary would be the host (human) if this mechanism could help to keep a healthy balanced microbiota and therefore help to maintain a state of well-being.

In the current state of the art, several critical points should be addressed to confirm or discard the previously set up hypothesis (Figure 4). Apart from some general points that need to be defined in the prebiotic research field, such as, for example, the definition of the target microbial groups, the specific needs on research about bacterial EPS as prebiotics should mainly focus at (i) establishing differential physicochemical properties are intrinsic to each biopolymer that confer susceptibility of biodegradation, and (ii) identifying the enzymatic machinery (glycosyl hydrolase) harbored by the intestinal microbiota which have not been described up to date. Furthermore, the pathways of transport and metabolism of the released monosaccharides and oligosaccharides from the high molecular weight bacterial EPS remain to be elucidated, as well as the putative cross-feeding mechanisms among microbiota members. Moreover, as far as we know, no human intervention trials have been carried out to demonstrate the prebiotic capability of these bacterial origin biopolymers. Finally, regarding the application of LAB-EPS or bifido-EPS as potential prebiotics for food technology and human nutrition, it is necessary to underline the limitation of the use of these polymers due to the scarce production yield using rich (and expensive) culture media. However, a promising approach for future applications in the functional food area could be the use of the EPS-producing strains, thus combining their potential as probiotics and prebiotics, in a kind of "synbiotic" ingredient. But we need to keep in mind that human studies are still needed to confirm or discard the capability of bacterial EPS to act as modulators of the intestinal microbiota, and therefore to associate this modulation with a beneficial effect for the host.

FUNDING

The authors are grateful to the financial support given to this research topic by the (currently) Ministry of Economy and Competiveness (MINECO) throughout European Union FEDER funds. The acknowledged ongoing projects of the group are AGL2009-09445 and AGL2010-16525.

REFERENCES

Arboleya, S., González, S., Salazar, N., Ruas-Madiedo, P., de los Reyes-Gavilán, C. G. and Gueimonde, M. (2012). Development of probiotic products for nutritional requirements of specific human populations. *Eng. Life Sci.* **12**:368–376.

Arumugam, M., Raes, J., Pelletier, E., Le Paslier, D., Yamada, T., Mende, D. R., Fernandes, G. R., Tap, J., Bruls, T., Batto, J. M., Bertalan, M., Borruel, N., Casellas, F., Fernandez, L., Gautier, L., Hansen, T., Hattori, M., Hayashi, T., Kleerebezem, M., Kurokawa, K., Leclerc, M., Levenez, F., Manichanh, C., Nielsen, H. B., Nielsen, T., Pons, N., Poulain, J., Qin, J., Sicheritz-Ponten, T., Tims, S., Torrents, D., Ugarte, E., Zoetendal, E. G., Wang, J., Guarner, F., Pedersen, O., de Vos, W. M., Brunak, S., Doré, J. and MetaHIT Consortium. (2011). Enterotypes of the human gut microbiome. *Nature*. **473**:174–180. [Erratum in: *Nature*. (2011). **474**:666]

Ashida, H., Ogawa, M., Kim, M., Mimuro, H. and Sasakawa, C. (2012). Bacteria and host interaction in the gut epithelial barrier. *Nat. Chem. Biol.* **8**:36–45.

Bäckhed, F., Ley, R. E., Sonnenburg, J. L., Peterson, D. A. and Gordon, J. I. (2005). Host-bacterial mutualism in the human intestine. *Science*. **307**:1915–1920.

Barrangou, R., Altermann, E., Hutkins, R., Cano, R. and Klaenhammer, T. R. (2003). Functional and comparative genomic analyses of an operon involved in fructooligosaccharide utilization by *Lactobacillus acidophilus*. *Proc. Natl Acad. Sci. USA*. **100**:8957–8962.

Behare, P. V., Singh, R., Kumar, M., Prajapati, J..B. and Singh, R. P. (2009). Exopolysaccharides of latic acid bacteria: A review. *J. Food Sci. Technol.* **46**:1–11.

Bergmaier, D., Champagne, C. P. and Lacroix, C. (2005). Growth and exopolysaccharide production during free and immobilized cell chemostat culture of *Lactobacillus rhamnosus* RW-9595M. *J. Appl. Microbiol.* **98**:272–284.

Biagi, E., Nylund, L., Candela, M., Ostan, R., Bucci, L., Pini, E., Nikkila, J., Monti, D., Satokari, R., Franceschi, C., Brigidi, P. and De Vos, W. (2010). Through ageing and beyond: Gut microbiota and inflammatory status of seniors and centenarians. *PLoS One*. **5**:e10667.

Bird, A. R., Conlon, M. A., Christophersen, C. T. and Topping, D. L. (2010). Resistant starch, large bowel fermentation and a broader perspective of prebiotics and probiotics. *Benef. Microbes.* **1**:423–431.

Broadbent, J. R., McMahon, D. J., Welker, D. L., Oberg, C. J. and Moineau, S. (2003). Biochemistry, genetics and applications of exopolysaccharide production in *Streptococcus thermophilus*: A review. *J. Dairy. Sci.* **86**:407–423.

Broekaert, W. F., Courtin, C. M., Verbeke, K., Van de Wiele, T., Verstraete, W. and Delcour, J. A. (2011). Prebiotic and other health-related effects of cereal-derived arabinoxylans, arabinoxylan-oligosaccharides, and xylooligosaccharides. *Crit. Rev. Food Sci. Nutr.* **51**:178–194.

Candela, M., Maccaferri, S., Turroni, S., Carnevali, P. and Brigidi, P. (2010). Functional intestinal microbiome, new frontiers in prebiotic design. *Int. J. Food Microbiol.* **140**:93–101.

Casci, T. and Rastall, R. A. (2006). Manufacture of prebiotic oligosaccharides. **In**: Prebiotics, Development and Application, pp. 29–56. Gibson, G. R. and Rastall, R. A., Eds., John Wiley, West Sussex, UK.

Cencic, A. and Langerholc, T. (2010). Functional cell models of the gut and their application in food microbiology – A review. *Int. J. Food Microbiol.* **141**:S4–S14.

Cescutti, P. (2009). Bacterial capsular polysaccharides and exopolysaccharides. **In**: Microbial Glycobiology, Structures, Relevance and Applications, pp. 93–108. Moran, A. P., Holst, O., Brennan, P. J. and von Itzstein, M., Eds., Academic Press, Elsevier, London.

Charalampopoulos, D. and Rastall, R. A. (2012). Prebiotics in foods. *Curr. Opin. Biotechnol.* **23**:197–191.

Chung, C. H. and Day, D. F. (2002). Glucooligosaccharides from *Leuconostoc mesenteroides* B-742 (ATCC 13146): A potential prebiotic. *J. Indus. Microbiol. Biotechnol.* **29**:196–199.

Cinquin, C., Le Blay, G., Fliss, I. and Lacroix, C. (2006). Comparative effects of exopolysaccharides from lactic acid bacteria and fructo-oligosaccharides on infant gut microbiota tested in an in vitro colonic model with immobilized cells. *FEMS Microbiol. Ecol.* **57**:226–238.

Crittenden, R. and Playne, M. J. (2009). Prebiotics. **In**: Handbook of Probiotics and Prebiotics, 2nd ed., pp. 535–562. Lee, Y. K. and Salminen, S., Eds., Wiley Interscience, New York, NY.

Cummings, J. H., Antoine, J. M., Azpiroz, F., Bourdet-Sicard, R., Brandtzaeg, P., Calder, P. C., Gibson, G. R., Guarner, F., Isolauri, E., Pannemans, D., Shortt, C., Sandra, S., Tuijtelaars, S. and Watzl, B. (2004). PASSCLAIM-Gut health and immunity. *Eur. J. Nutr.* **43**:118–173.

Dal Bello, F., Walter, J., Hertel, C. and Hammes, W. P. (2001). *In vitro* study of prebiotic properties of levan-type exopolysaccharides from lactobacilli

and non-digestible carbohydrates using denaturing gradient gel electrophoresis. *System. Appl. Microbiol.* **24**:232–237.

De Filippo, C., Cavalieri, D., Di Paola, M., Ramazzotti, M., Poullet, J. B., Massart, S., Collini, S., Pieraccini, G. and Lionetti, P. (2010). Impact of diet in shaping gut microbiota revealed by a comparative study in children from Europe and rural Africa. *Proc. Natl Acad. Sci. USA*. **107**:14691–14696.

de los Reyes-Gavilán, C. G., Suárez, A., Fernández-García, M., Margolles, A., Gueimonde, M. and Ruas-Madiedo, P. (2011). Adhesion of bile-adapted *Bifidobacterium* strains to the HT29-MTX cell line is modified after sequential gastrointestinal challenge simulated in vitro using human gastric and duodenal juices. *Res. Microbiol.* **162**:514–519.

De Vuys, L., de Vin, F., Vaningelgem, F. and Degeest, B. (2001). Recent development in the biodsynthesis and applications of heteropolysaccharides from lactic acid bacteria. *Int. Dairy J*. **11**:687–707.

De Vuyst, L. and Leroy, F. (2011). Cross-feeding between bifidobacteria and butyrate-producing colon bacteria explains bifdobacterial competitiveness, butyrate production, and gas production. *Int. J. Food Microbiol.* **149**:73–80.

Deggest, B., Vaningelgem, F. and De Vuyst, L. (2001). Microbial physiology, fermentation kinetics and process engineering of heteropolysaccharide production by lactic acid bacteria. *Int. Dairy J*. **11**:747–757.

Delzene, N. M. and Kok, N. (2001). Effects of fructan-type prebiotics on lipid metabolism. *Am. J. Clin. Nutr.* **73**:456–458.

Denou, E., Pridmore, R. D., Berger, B., Panoff, J. M., Arigoni, F. and Brüssow, H. (2008). Identification of genes associated with the long-gut persistence phenotype of the probiotic *Lactobacillus johnsonii* strain NCC533 using a combination of genomic and transcriptomic analysis. *J. Bacteriol.* **190**:3161–3168.

Dethlefsen, L. and Relman, D. A. (2011). Incomplete recovery and individualized responses of the human distal gut microbiota to repeated antibiotic perturbation. *Proc. Natl Acad. Sci. USA*. **108**(Suppl. 1):4554–4561.

DuPont, A. W. and DuPont, H. L. (2011). The intestinal microbiota and chronic disorders of the gut. *Nat. Rev. Gastroenterol. Hepathol.* **8**:523–531.

Falk, P. G., Hooper, L. V., Midvedt, T. and Gordon, J. I. (1998). Creating and maintaining the gastrointestinal ecosystem: What we know and need to know from gnotobiology. *Microbiol. Mol. Biol. Rev.* **62**:1157–1170.

Fanning, S., Hall, L. J., Cronin, M., Zomer, A., McSharry, J., Goulding, D., O'Connell-Motherway, M., Shanahan, F., Nally, K., Dougan, G. and van Sinderen, D. (2012). Bifidobacterial surface-exopolysaccharide facilitates commensal-host interaction through immune modulation and pathogen protection. *Proc. Natl Acad. Sci. USA*. **109**:2108–2113.

FAO/WHO. (2006). Probiotics in food. Health and nutritional properties and guidelines for evaluation. FAO Food and Nutritional Paper No. 85 (ISBN 92–5–105513–0).

FAO/WHO. (2007). FAO Technical Meeting on Prebiotics. WHO, Geneva, Switzerland. http.//www.fao.org/ag/agn/agns/files/Prebiotics_Tech_Meeting_Report.pdf.

Flint, H. J. (2012). The impact of nutrition on the human microbiome. *Nutr. Rev.* **70**:S10–S13.

Flint, H. J., Bayer, E. A., Rincon, M. T., Lamed, R. and White, B. A. (2008). Polysaccharide utilization by gut bacteria: Potential for new insights from genomic analysis. *Nature Rev. Microbiol.* **6**:121–131.

Fraher, M. H., O'Toole, P. W. and Quigley, E. M. M. (2012). Techniques used to characterize the gut microbiota: A guide for the clinician. *Nature Rev. Gastroenterol. Hepatol.* **9**:312–322.

Fukuda, S., Toh, H., Hase, K., Oshima, K., Nakanishi, Y., Yoshimura, K., Tobe, T., Clarke, J. M., Topping, D. L., Suzuki, T., Taylor, T. D., Itoh, K., Kikuchi, J., Morita, H., Hattori, M. and Ohno, H. (2011). Bifidobacteria can protect from enteropathogenic infection through production of acetate. *Nature*. **469**:543–549.

Gänzle, M. and Schwab, C. (2009). Ecology of exopolysaccharide formation by lactic acid bacteria: Sucrose utilization, stress tolerance and biofim formation. **In**: Bacterial Polysaccharides, Current Innovations and Future Trends, pp. 263–278. Ullrich, M., Ed., Caister Academic Press, Norfolk, UK.

Gao, Z., Yin, J., Zhang, J., Ward, R. E., Martin, R. J., Lefevre, M., Cefalu, W. T. and Ye, J. (2009). Butyrate improves insulin sensitivity and increases energy expenditure in mice. *Diabetes*. **58**:1509–1517.

Gibson, G. R. and Roberfroid, M. B. (1995). Dietary modulation of the human colonic microbiota: Introducing the concept of prebiotics. *J. Nutr.* **125**:1401–1412.

Gibson, G. R., Probert, H. M., Van Loo, J., Rastall, R. A. and Roberfroid, M. B. (2004). Dietary modulation of the human colonic microbiota: Updating the concept of prebiotics. *Nutr. Res. Rev.* **17**:259–275.

Hassan, A. N. (2008). Possibilities and challenges of exopolysaccharide-producing lactic cultures in dairy foods. *J. Dairy Sci.* **91**:1282–1298.

Hidalgo-Cantabrana, C., López, P., Gueimonde, M., de los Reyes-Gavilán, C. G., Suárez, A., Margolles, A. and Ruas-Madiedo, P. (2012). Immune modulation capability of exopolysaccharides synthesized y lactic acid bacteria and bifidobacteria. *Probiotics Antimicro. Prot.* **4**:227–237.

Hongpattarakere, T., Cherntong, N., Wichienchot, S., Kolida, S. and Rastall, R. A. (2012). *In vitro* prebiotic evaluation of exopolysaccharides produced by marine isolated lactic acid bacteria. *Carbohyd. Pol.* **87**:846–852.

Hyötyläinen, T. (2012). Novel methodologies in metabolic profiling with a focus on molecular diagnostic applications. *Expert Rev. Mol. Diagn.* **12**:527–538.

International Association of Probiotics and Prebiotics (ISAPP). (2008). 6th Meeting of the International Scientific Association of Probiotics and Prebiotics, London, Ontario, Canada.

Jolly, L. and Stingele, F. (2001). Molecular organization and functionality of exopolysaccharide gene cluster in lactic acid bacteria. *Int. Dairy J*. **11**:733–745.

Koenig, J. E., Spor, A., Scalfone, N., Fricker, A. D., Stombaugh, J., Knight, R., Angenent, L. T. and Ley, R. E. (2011). Succession of microbial consortia in the developing infant gut microbiome. *Proc. Natl Acad. Sci. USA*. **108** (Suppl. 1):4578–4585.

Korakli, M. and Vogel, R. F. (2006). Structure/function relationship of homopolysaccharide producing glycansucrases and therapeutic potential for their synthesized glycans. *Appl. Microbiol. Biotechnol.* **71**:790–803.

Korakli, M., Gänzle, M. G. and Vogel, R. F. (2002). Metabolism by bifidobacteria and lactic acid bacteria polysaccharides from wheat and rye, and exopolysaccharides produced by *Lactobacillus sanfranciscensis*. *J. Appl. Microbiol.* **92**:958–965.

Lebeer, S., Claes, I. J. J., Verhoeven, T. L. A., Vanderleyden, J. and De Keersmaecker, S. C. J. (2010). Exopolysaccharide of *Lactobacillus rhamnosus* GG form a protective shield against innate immune factors in the intestine. *Microbial Biotechnol.* **4**:368–374.

Leivers, S., Hidalgo-Cantabrana, C., Robinson, G., Margolles, A., Ruas-Madiedo, P. and Laws, P. L. (2011). Structure of the high molecular weight exopolysaccharide produceb by *Bifidobacterium animalis* subsp. *lactis* IPLA-R1 and sequence anlaysis of its putative *eps* cluster. *Carbohydr. Res.* **346**:2710–2717.

Leroy F. and De Vuyst, L. (2004). Lactic acid bacteria as functional starter cultures for food fermentation industry. *Tren. Food Sci. Technol.* **15**:67–78.

Looijesteijn, P. J., Trapet, L., de Vries, E., Abee, T. and Hugenholtz, J. (2001). Physiological function of exopolysaccharides produced by *Lactococcus lactis*. *Int. J. Food Microbiol.* **64**:71–80.

López, P., Monteserín, D. C., Gueimonde, M., de los Reyes-Gavilán, C. G., Margolles, A., Suárez, A. and Ruas-Madiedo, P. (2012). Exopolysaccharide-producing *Bifidobacterium* strains elicit different in vitro responses upon interaction with human cells. *Food Res. Int.* **46**:99–107.

Margolles, A., Mayo, B. and Ruas-Madiedo, P. (2009). Screening, identification and characterization of Lactobacillus and Bifidobacterium strains. **In**: Handbook of Probiotics and Prebiotics, 2nd ed., pp. 4–24. Lee, Y. K. and Salminen, S., Eds., Wiley Interscience, New York, NY.

Martensson, O., Bifrklund, M., Lambo, A. M., Dueñas-Chasco, M., Irastorza, A., Holst, O., Norin, E., Welling, G., Öste, R. and Önning, G. (2005). Fermented, ropy, oat-based products reduce cholesterol levels and stimulate the bifidobacteria flora in humans. *Nutr. Res.* **25**:429–442.

Marzorati, M., Van Den Abbeele, P., Possemiers, S., Benner, J., Verstraete, W. and Van De Wiele, T. (2011). Studying the host-microbiota interaction in the human gastrointestinal tract: Basic concepts and *in vitro* approaches. *Ann. Microbiol.* **61**:709–715.

Monsan, P., Bozonnet, S., Albenne, C., Joucla, G., Willemot, R. M. and Remaud-Siméon, M. (2001). Homopolysaccharides from lactic acid bacteria. *Int. Dairy J*. **11**:675–685.

Mozzi, F., Gerbino, E., Font de Valdez, G. and Torino, M. I. (2009). Functionality of exopolysaccharides produced by lactic acidbacteria in an in vitro gastric system. *J Appl Microbiol.* **107**:56–64.

Mozzi, F., Vaningelgem, F., Hébert, E. M., van der Meulen, R., Foulquié-Moreno, M. R., Font de Valdez, G. and De Vuyst, L. (2006). Diversity of heteropolysaccharide producing lactic acid bacterium strains and their biopolymers. *Appl. Environ. Microbiol.* **72**:4431–4435.

Mueller, S., Saunier, K, Hanisch, C., Norin, E., Alm, L., Midtvedt, T., Cresci, A., Silvi, S., Orpianesi, C., Verdenelly, M. C., Clavel, T., Koebrick, C., Zunft, H. H., Doré, J. and Blaut, M. (2006). Differences in fecal microbiota in different European study populations in relation to age, gender, and country: A cross-sectional study. *Appl. Environ. Microbiol.* **72**:1027–1033.

Nikolic, M., López, P., Strahinic, I., Suárez, A., Kojic, M., Fernández-García, M., Topisirovic, L., Golic, N. and Ruas-Madiedo, P. (2012). Characterisation of the exopolysaccharide (EPS)-producing *Lactobacillus paraplantarum* BGCG11 and its non-EPS producing derivative strains as potential probiotics. *Int. J. Food Microbiol.* **158**:155–162.

Nugent, A. P. (2005). Health properties of resistant starch. *Nutr. Bull.* **30**: 27–54.

O'Connell-Motherway, M., Zomer, A., Leahy, S. C., Reunanen, J., Bottacini, F., Claesson, M. J., O'Brien, F., Flynn, K., Casey, P. G., Muñoz, J. A., Kearney, B., Houston, A. M., O'Mahony, C., Higgins, D. G., Shanahan, F., Palva, A., de Vos, W. M., Fitzgerald, G. F., Ventura, M., O'Toole, P. W. and van Sinderen, D. (2011). Functional genome analysis of *Bifidobacterium breve* UCC2003 reveals type IV tight adherence (Tad) pili as an essential and conserved host-colonization factor. *Proc. Natl Acad. Sci. USA*. **108**:11217–11222.

Peng, L., Li, Z-R., Green, R. S., Holzman, I. R. and Lin, J. (2009). Butyrate enhances the intestinal barrier by facilitating tight junction assembly via activation of AMP-activated protein kinase in Caco-2 cell monolayers. *J. Nutr*. **139**:1619–1625.

Peso-Echarri, P., Marínez-Graciá, C., Ros-Berruezo, G., Vives, I., Ballesta, M., Solís, G., Vasallo-Morillas, I., de los Reyes-Gavilán, C. G., Margolles, A. and Gueimonde, M. (2011). Assessment of intestinal microbiota of full-term breast-fed infants from two different geographical locations. *Early Hum. Dev.* **87**:511–513.

Qin, J., Li, R., Raes, J., Arumugam, M., Burgdorf, K. S., Manichanh, C., Nielsen, T., Pons, N., Levenez, F., Yamada, T., Mende, D. R., Li, J., Xu, J., Li, S., Li, D., Cao, J., Wang, B., Liang, H., Zheng, H., Xie, Y., Tap, J., Lepage, P., Bertalan, M., Batto, J. M., Hansen, T., Le Paslier, D., Linneberg, A., Nielsen, H. B., Pelletier, E., Renault, P., Sicheritz-Ponten, T., Turner, K., Zhu, H., Yu, C., Li, S., Jian, M., Zhou, Y., Li, Y., Zhang, X., Li, S., Qin, N., Yang, H., Wang, J., Brunak, S., Doré, J., Guarner, F., Kristiansen, K., Pedersen, O., Parkhill, J., Weissenbach, J. and MetaHIT Consortium. (2010). A human gut microbial gene catalogue established by metagenomic sequencing. *Nature*. **464**:59–65.

Roberfroid, M. B., Gibson, G. R., Hoyles, L., McCartney, A. L., Rastall, R., Rowland, I., Wolvers, D., Watzl, B., Szajewska, H., Stahl, B., Guarner, F., Respondek, F., Whelan, K., Coxam, V., Davicco, J. J., Létoing, L, Wittrant, J., Delzenne, N. M., Cani, P. D., Neyrinck, A. M. and Meheust, A. (2010). Prebiotic effects: Metabolic and health benefits. *Br. J. Nutr.* **104**:S1–S63.

Rossi, M., Corradini, C., Amaretti, A., Nicolini, M., Pompei, A., Zanoni, S. and Matteuzzi, D. (2005). Fermentation of fructooligosaccharides and inulin by bifidobacteria: A comparative study of pure and fecal cultures. *Appl. Environ. Microbiol.* **71**:6150–6158.

Round, J. L. and Mazmanian, S. K. (2009). The gut microbiota shapes intestinal immune responses during health and disease. *Nat. Rev. Immunol.* **9**:313–323.

Ruas-Madiedo, P., Moreno, J. A., Salazar, N., Delgado, S., Mayo, B., Margolles, A. and de los Reyes-Gavilán, C. G. (2007). Screening of exopolysaccharide-producing *Lactobacillus* and *Bifidobacterium* strains isolated from the human intestinal microbiota. *Appl. Environ. Microbiol.* **73**:4385–4388.

Ruas-Madiedo, P., Salazar, N. and de los Reyes-Gavilán, G. C. (2009a). Biosynthesis and chemical composition of exopolysaccharides produced by lactic acid bacteria. **In**: *Bacterial Polysaccharides, Current Innovations and Future Trends*, pp. 279–310. Ullrich, M., Ed., Caister Academic Press, Norfolk, UK.

Ruas-Madiedo, P., Salazar, N. and de los Reyes-Gavilán, G. C. (2009b). Exopolysaccharides produced by lactic acid bacteria in food and probiotic applications. **In**: Microbial Glycobiology, Structures, Relevance and Applications, pp. 887–902. Moran, A. P., Holst, O., Brennan, P. J. and von Itzstein, M., Eds., Academic Press, London.

Ruas-Madiedo, P., Sánchez, B., Hidalgo-Cantabrana, C., Margolles, A. and Laws, A. (2012). Exopolysaccharides from lactic acid bacteria and bifidobacteria. **In**: Handbook of Animal-Based Fermented Food and Beverage Technology, 2nd ed., pp. 125–151. Hui, Y. H. and Evranuz, E. O., Eds., CRC Press, Boca Raton, FL.

Ruijssenaars, H. J., Stingele, F. and Hartmans, S. (2000). Biodegradability of food-associated extracellular polysaccharides. *Curr. Microbiol.* **40**:194–199.

Salazar, N., Binetti, A., Gueimonde, M., Alonso, A., Garrido, P., González del Rey, C., González, C., Ruas-Madiedo, P. and de los Reyes-Gavilán, C. G. (2011). Safety and intestinal microbiota modulation by the exopolysaccharide-producing strains *Bifidobacterium animalis* IPLA R1 and *Bifidobacterium longum* IPLA E44 orally administered to Wistar rats. *Int. J. Food Microbiol.* **144**:342–351.

Salazar, N., Gueimonde, M., Hernańdez-Barranco, A. M., Ruas-Madiedo, P. and de los Reyes-Gavilán, C. G. (2008). Exopolysaccharides produced by intestinal *Bifidobacterium* strains act as fermentable substrates for human intestinal bacteria. *Appl. Environ. Microbiol.* **74**:4737–4745.

Salazar, N., Prieto, A., Leal, J. A., Mayo, B., Bada-Gancedo, J. C., de los Reyes-Gavilán, C. G. and Ruas-Madiedo, P. (2009a). Production of exopolysaccharides by *Lactobacillus* and *Bifidobacterium* strains of human origin, and metabolic activity of the producing bacteria in milk. *J. Dairy Sci.* **92**:4158–4168.

Salazar, N., Ruas-Madiedo, P., Kolida, S., Collins, M., Rastall, R. A., Gibson, G. R. and de los Reyes-Gavilan, C. G. (2009b). Exopolysaccharides produced by *Bifidobacterium longum* IPLA E44 and *Bifidobacterium animalis* subsp. *lactis* IPLA R1 modify the composition and metabolic activity of human faecal microbiota in pH-controlled batch cultures. *Int. J. Food Microbiol.* **135**:260–267.

Salminen, S. and Gueimonde, M. (2005). Gut microbiota in infants between 5 and 24 months of age. **In**: Feeding During Late Infancy and Early Childhood: Impact on Health. Nestle Nutrition Workshop Series Pediatric Program. **56**, pp. 43–51. Hernell, O. and Schmitz, J. Eds. Nestec, Basel, Switzerland.

Sarbini, S. R., Kolida, S., Naeye, T., Einerhand, A., Brison, Y., Remaud-Simeon, M., Monsan, P., Gibson, G. R. and Rastall, R. A. (2011). *In vitro* fermentation of linear and α-1,2-branched dextrans by the human fecal microbiota. *Appl. Environ. Microbiol.* **77**:5307–5315.

Saulnier, D. M. L., Kolida, S. and Gibson, G. R. (2009). Microbiology of the human intestinal tract and approaches for its dietary modulation. *Curr. Parm. Des.* **15**:1403–1414.

Schell, M. A., Karmirantzou, M., Snel, B., Vilanova, D., Berger, B., Pessi, G., Zwahlen, M-C., Desiere, F., Bork, P., Delley, M., Pridmore, R. D. and Arigoni, F. (2002). The genome sequence of *Bifidobacterium longum* reflects its adaptation to the human gastrointestinal tract. *Proc. Natl Acad. Sci. USA*. **99**:14422–14427.

Sekirov, I., Russell, S. L., Antunes, L. C. and Finlay, B. B. (2010). Gut microbiota in health and disease. *Physiol. Rev.* **90**:859–904.

Sela, D. A. and Mills, D. A. (2010). Nursing our microbiota: molecular linkages between bifidobacteria and milk oligosaccharides. *Trends Microbiol.* **18**:298–307.

Sela, D. A., Chapman, J., Adeuya, A., Kim, J. H., Chen, F., Whitehead, T. R., Lapidus, A., Rokhsar, D. S., Lebrilla, C. B., German, J. B., Price, N. P., Richardson, P. M. and Mills, D. A. (2008). The genome sequence of *Bifidobacterium longum* subsp. *infantis* reveals adaptations for milk utilization within the infant microbiome. *Proc. Natl Acad. Sci. USA*. **105**:18964-18969.

Sonnenburg, J. L., Xu, J., Leip, D. D., Chen, C. H., Westover, B. P., Weatherford, J., Buhler, J. D. and Gordon, J. I. (2005). Glycan foraging *in vivo* by an intestine-adapted bacterial symbiont. *Science*. **307**:1955–1959.

Tabernero, M., Venema, K., Maathuis, A. J. H. and Saura-Calixto, F. D. (2011). Metabolite production during *in vitro* colonic fermentation of dietary fiber: Analysis and comparison of two European diets. *J. Agric. Food Chem.* **59**:8968–8975.

Tieking, M., Kaditzky, S., Valcheva, R., Korakli, M., Vogel, R. F. and Gänzle, M. G. (2005). Extracellular homopolysaccharides and oligosaccharides from intestinal lactobacilli. *J. Appl. Microbiol.* **99**:692–702.

Tremaroli, V. and Bäckhed, F. (2012). Functional interactions between the gut microbiota and host metabolism. *Nature.* **489**:242–249.

Turnbaugh, P. J. and Gordon, J. I. (2009). The core gut microbiome, energy balance, and obesity. *J. Physiol.* **587**:4153–4158.

Turroni, F., Bottacini, F., Foroni, E., Mulder, I., Kim, J. H., Zomer, A., Sánchez, B., Bidossi, A., Ferrarini, A., Giubellini, V., Delledonne, M., Henrissat, B., Coutinho, P., Oggioni, M., Fitzgerald, G. F., Mills, D., Margolles, A., Kelly, D., van Sinderen, D. and Venura, M. (2010). Genome analysis of *Bifidobacterium bifidum* PRL2010 revels metabolic pathways for host-derived glycan foraging. *Proc. Natl Acad. Sci. USA*. **107**:19514–9.

Van Calsteren, M. R., Pau-Roblot, C., Begin, A. and Roy, D. (2002). Structure determination of the exopolysaccharide produced by *Lactobacillus rhamnosus* strains RW-9595M and R. *Biochem. J*. **363**:7–17.

Van de Wiele, T., Boon, N., Possemiers, S., Jacob, H. and Verstraete, W. (2007). Inulin-type fructans of longer degree of polymerization exert more pronounced *in vitro* prebiotic effects. *J. Appl. Microbiol.* **102**: 452–460.

Van den Abbeele, P., Grootaert, C., Marzorati, M., Possemiers, S., Verstraete, W., Gérard, P., Rabot, S., Bruneau, A., El Aidy, S., Derrien, M., Zoetendal, E., Kleerebezem, M., Smidt, H. and Van de Wiele, T. (2010). Microbial community development in a dynamic gut model is reproducible, colon region specific, and selective for Bacteroidetes and Clostridium cluster IX. *Appl. Environ. Microbiol.* **76**:5237–5246.

Van den Abbeele, P., Roos, S., Eeckhaut, V., MacKenzie, D. A., Derde, M., Verstraete, W., Marzorati, M., Possemiers, S., Vanhoecke, B., van Immerseel, F. and van de Wiele, T. (2012). Incorporating a mucosal environment in a dynamic gut model results in a more representative colonization by lactobacilli. *Microb. Biotechnol.* **5**:106–115.

Van Geel-Schutten, G. H., Faber, E. J., Smit, E., Monting, K., Smith, M. R., Ten Brink, B., Kamerling, J. P., Vliegenthart, J. F. G. and Dijkhuizen, L. (1999). Biochemical and structural characterization of the glucan and fructan expolysaccharides synthesized by the *Lactobacillus reuteri* wild-type strain and by mutant strains. *Appl. Environ. Microbiol.* **65**:3008–3014.

Van Hijum, S. A. F. T., Kralj, S., Ozimek, L. K., Dijkhuizen, L. and van Geel-Schutten, I. G. H. (2006). Structure-function relationships of glucansucrase and fructansucrase enzymes from lactic acid bacteria. *Microbiol. Mol. Bio. Rev.* **70**:157–176.

Vrieze, A., Holleman, F., Zoetendal, E. G., de Vos, W. M., Hoekstra, J. B. L. and Nieuwdorp, M. (2010). The environment within: How gut microbiota may influence metabolism and body composition. *Diabetologia.* **53**:606–613.

Werning, M. L., Ibarburu, I., Dueñas, M. T., Irastorza, A., Navas, J. and López, P. (2006). *Pediococcus parvulus gtf* gene encoding the GTF glycosyltransferase and its application for specific PCR detection of β-D-glucan-producing bacteria in foods and beverages. *J. Food Prot.* **69**:161–169.

Xiao, J. Z., Takahashi, S., Nishimoto, M., Odamaki, T., Yaeshima, T., Iwatsuki, K. and Kitaoka, M. (2010). Distribution of *in vitro* fermentation ability of lacto-N-biose I, a major building block of human milk oligosaccharides, in bifidobacterial strain. *Appl. Environ. Microbiol.* **76**:54–59.

Yu, J., Peng, S., Luo, D. and March, J. C. (2012). *In vitro* 3D human small intestinal villous model for drug permeability determination. *Biotechnol. Bioeng.* **109**:2173–2178.