

# Genomic Overview and Biological Functions of Exopolysaccharide Biosynthesis in *Bifidobacterium* spp.

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For many years, bacterial exopolysaccharides (EPS) have received considerable scientific attention, mainly due to their contribution to biofilm formation and, above all, because EPS are potential virulence factors. In recent times, interest in EPS research has enjoyed a welcome boost thanks to the discovery of their ability to mediate communication processes with their surrounding environment and to their contribution to host health maintenance. In this review, we provide a fresh perspective on the genetics and activity of these polymers in members of the *Bifidobacterium* genus, a common gut inhabitant of humans and animals that has been associated with several health-promoting effects. Bifidobacteria can use EPS to protect themselves against the harsh conditions of the gastrointestinal tract, thus improving their persistence in the host. Indeed, the relevant function of EPS for bifidobacteria is underlined by the fact that most genomes sequenced until now contain genes related to EPS biosynthesis. A high interspecies variability in the number of genes and structural organization is denoted among species/subspecies; thus, *eps* clusters in this genus do not display a consensus genetic architecture. Their different G+C content compared to that of the whole genome suggests that *eps* genes have been acquired by horizontal transfer. From the host perspective, EPS-producing bifidobacteria are able to trigger both innate and adaptive immune responses, and they are able to modulate the composition and activity of the gut microbiota. Thus, these polymers seem to be critical in understanding the physiology of bifidobacteria and their interaction with the host.

Exopolysaccharides (EPS) are carbohydrate polymers present as an extracellular layer covering the surface of a vast variety of microorganisms, including Gram-positive as well as Gram-negative bacteria. Interest in this research topic comes from the potential applications of EPS in diverse sectors, such as food, biotechnology, cosmetics, or medicine (1, 2), as well as from their implications for health. Some EPS have been reported to act as virulence factors in several infectious diseases (3, 4), but, in contrast, it has also been shown that bacterial EPS can have a positive impact on human health (5, 6). Indeed, the production of EPS by some bacteria with probiotic traits has been tentatively correlated with their capability to modulate the host immune response, eliciting both innate and adaptive responses (7–10). Additionally, EPS also help the producing bacteria, when they are ingested, to overcome gastrointestinal challenges and to persist for longer periods in the gut (8, 11). It has been shown that bile salts induced the synthesis of EPS in some potentially probiotic bacteria (12, 13). Therefore, it seems that these polymers could play a relevant role in the cross talk between the EPS-producing bacteria and the gut environment of the host.

One of the most cited definitions of probiotics is “live microorganisms which when administered in adequate amounts confer a health benefit on the host” (14). The commonly incorporated bacteria in probiotic foods or supplements for human consumption belong to the genera *Bifidobacterium* and *Lactobacillus*. Members of the two genera are Gram positive and are separated into different phyla according to their G+C content. Lactobacilli, included in the phylum *Firmicutes*, are non-spore-forming rods or coccobacilli with low G+C content (<50%), whereas bifidobacteria are members of *Actinobacteria* (G+C > 50%) and are non-filamentous rods which can display different morphologies, the bifurcated or “bifido” shape being the most common. Both genera naturally inhabit common ecosystems, such as the oral cavity and

the gastrointestinal tract of different animals (15, 16). Furthermore, lactobacilli are naturally present in different fermented foods since they are present as contaminants of raw agricultural materials. Bifidobacteria normally appear in foods only when they are added during the formulation of probiotic products (17). EPS production has been extensively studied in *Lactobacillus* and other genera of lactic acid bacteria (LAB) of food origin. This topic has been the subject of several reviews, each with a different focus (for example, see references 18, 19, and 20). Some of the EPS-producing LAB are used in the dairy industry to improve the sensorial properties of fermented products since their polymers act as natural biothickeners, fat replacers, and viscosity intensifiers (21, 22). The genetics of EPS synthesis in food lactobacilli and other LAB has also been reviewed (23–25). The interest in studying EPS biosynthesis in lactobacilli and bifidobacteria of animal origin is more recent and comes from their biological properties, as EPS are involved in the interactions with the host (20, 26, 27). However, there is little information concerning this anabolic route in bifidobacteria. Therefore, after a short summary of the biological functions attributed to bifidobacterial EPS (bifido-EPS), in this review we aim at providing an overview of the EPS biosynthesis pathway in this genus.

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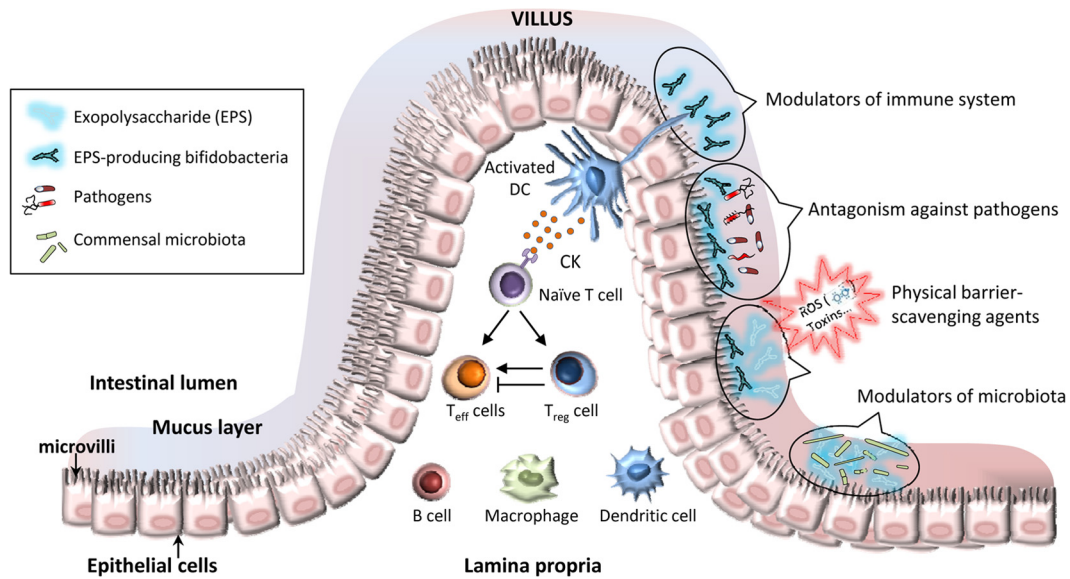


FIG 1 Beneficial activities potentially attributed to some exopolysaccharides synthesized by *Bifidobacterium*. CK, cytokines; DC, dendritic cell; T<sub>eff</sub> cells, lymphocyte T effector cells; T<sub>reg</sub> cell, lymphocyte T regulatory cell; B cell, lymphocyte B cell; ROS, reactive oxygen species.

### BIOACTIVITY OF EPS FROM *BIFIDOBACTERIUM*

As previously mentioned, the renewed interest in studying the synthesis of EPS in bifidobacteria is due to the role that these biopolymers could have in human health. Some *in vitro* studies have suggested that specific EPS can play a key role in the beneficial effect of the EPS-producing bifidobacteria. Nevertheless, it is worth pointing out the clear need for more research on this topic as well as for *in vivo* demonstration of such EPS-dependent biological activities. Figure 1 summarizes the pathways and cells that are involved in the effects EPS might have on the intestinal mucosa environment. Most reported studies were focused on the ability of polymers from LAB to modulate immune response (reviewed in reference 26). This is also a common characteristic found in some bifido-EPS, but only a few papers have dealt with this research topic. Using an *in vitro* approach, López and coworkers (9) have analyzed the immune activity of EPS purified from 13 strains belonging to three *Bifidobacterium* species (*B. animalis* subsp. *lactis*, *B. longum*, and *B. pseudocatenulatum*). Polymers, and some of the producing strains, were cocultivated with human peripheral blood mononuclear cells (PBMC), and pro- and anti-inflammatory cytokines were analyzed. They detected that the *in vitro* ability of these EPS to induce T cell differentiation with respect to Th1 (high tumor necrosis alpha [TNF- $\alpha$ ]/interleukin-10 [IL-10] ratio) or Th2 (high IL-10/IL-12 ratio) was highly dependent on the type of polymer. One remarkable finding that could shed light on some important traits that effector EPS might have is the case of the polymers purified from isogenic *B. animalis* subsp. *lactis* strains A1, A1dOx, and A1dOxR (also known as IPLA-R1) (28). The three polymers elicited different cytokine patterns, and the A1dOxR polymer, which has the highest molar mass, showed the lowest capability to induce the release of both pro- and anti-inflammatory cytokines by PBMC (9). Thus, it seems that large (high-molecular-weight [HMW]) polymers from bifidobacteria are not very efficient at inducing any immune response, just as was previously denoted for LAB-EPS (26). Fanning and coworkers (8) reported similar findings using *B. breve* UCC2003, with a model of

an EPS-producing (EPS<sup>+</sup>) strain and EPS-deficient (by insertion or deletion) derivative (EPS<sup>-</sup>) strains. In this case, *in vitro* cultures of splenocytes isolated from naive mice were cocultivated with the isogenic strains. The EPS<sup>+</sup> stimulated cells produced significantly lower levels of proinflammatory cytokines than those stimulated with the EPS<sup>-</sup> strains. When splenocytes isolated from treated mice (orally fed with EPS<sup>+</sup> or EPS<sup>-</sup> strains) were analyzed, it was confirmed that in the absence of the EPS, the number and activity of different immune cells increased with respect to the EPS<sup>+</sup>-fed mice or the naive mice. Therefore, it seems that the EPS-producing strain was not able to elicit a strong immune response compared with the EPS-deficient mutants. Apparently, the presence of the polymer surrounding the surface of *B. breve* UCC2003 could reduce the immune clearance of this bacterium due to a reduced B cell response and, therefore, this polymer may be crucial for the persistence of *B. breve* in the murine host. Furthermore, the presence of this EPS<sup>+</sup> strain in the murine intestine reduced the colonization by the pathogen *Citrobacter rodentium*, a result that was not promoted by the EPS<sup>-</sup> strain. Probably, as the authors suggested, this effect could have been mediated through the formation of a protective “bifidobacterial biofilm” provided by the EPS of *B. breve* (8). Similarly, Wu et al. (29) showed that the EPS purified from strain *B. longum* BCRC 1464 also showed antimicrobial activity against several pathogens. Besides, the cocultivation of macrophage J774A.1 cells with the heat-inactivated strain or with the purified EPS increased the release of anti-inflammatory IL-10; when cells were challenged with 100 ng/ml lipopolysaccharide (LPS), the purified EPS (at 5  $\mu$ g/ml) reduced the production of (proinflammatory) TNF- $\alpha$ . Those authors suggested that the EPS could contribute to the capability of *B. longum* to fight against gastrointestinal infections (29). Therefore, the presence of EPS could be another factor involved in the antagonism of bifidobacteria against pathogens. Our group has reported that purified EPS synthesized by *B. animalis* subsp. *lactis* increased the adhesion of certain enteropathogens to human mucus; this suggested that purified EPS were able to form an adherent layer on top

of mucus and that the bound EPS might have acted as ligands for molecules of the pathogen surface (30). Additionally, the polymers were able to counteract the cytotoxic effect of bacterial toxins (from *Bacillus cereus* and *Streptococcus pyogenes*) on colonocyte-like Caco2 cells and rabbit erythrocytes *in vitro* (31). Based on the results indicated above, we can hypothesize that the mechanism behind this antagonistic activity could be the creation of a physical barrier. Either the EPS-producing bacteria or the released polymer may act as a protective shield on the eukaryotic cells. Thus, the EPS layer could hinder the access of the pathogen, or its toxins, to the receptors in the surface of the eukaryotic cells. Similarly, the polymers could act as scavenging or trapping agents and, therefore, retain and remove these harmful agents from the intestinal niche (31). In any case, it seems that this nonspecific mechanism(s) could also be involved in the capability of some EPS-producing bifidobacteria to act as antioxidants, or free radical scavenging agents, to diminish the damage caused by reactive oxygen species in tissues (32). Regarding the host receptors that could be involved in the specific recognition of the bifidobacterial EPS, no information has been available in the literature to date. In any case, the molecules located at the surface of any microorganism are detected by pattern recognition receptors (PRR) of the host cells, such as Toll-like receptors (TLR), NOD-like receptors (NLR), and C-type lectin receptors (CLR). Some of these PRR are specific to glycan-type ligands, and they could be involved in the recognition of bacterial EPS (33, 34).

Several works have reported the suitability of different EPS synthesized by LAB to be used as fermentable substrates (or prebiotics) by the intestinal microbiota (reviewed in reference 27). In this scenario, our group studied this potential in bifido-EPS by means of *in vitro* human fecal-slurry models. To this end, 11 different polymers were separately analyzed in non-pH-controlled (35) or in pH-controlled (36) fecal batch cultures. In the presence of bifido-EPS, a modification in the diversity of certain bacterial populations and an increase in the number of some bifidobacterial species, as well as changes in the metabolic activity in the microbiota (measured in terms of short-chain fatty acid profiles), were noticeable. Therefore, it seems that bifido-EPS were able to feed specific groups of this fecal microbial community. However, it is worth noting that the use of these polymers as commercial prebiotic additives is very limited due to the low production yield by bifidobacteria. The idea of use of the whole EPS-producing bifidobacteria to modulate microbiota is more plausible.

From the works reviewed in this section, a corollary can be proposed: the physicochemical characteristics of EPS are directly related to their bioactivities. Thus, in order to find mechanisms of EPS activity, as well as to propose the best polymer targeted for specific applications, studying the genetics and the composition and structure of bifido-EPS is crucial. The current knowledge related to these topics is reviewed next.

#### EXOPOLYSACCHARIDES FROM *BIFIDOBACTERIUM* SPP.: STRUCTURE AND GENETICS

The EPS synthesized by bifidobacteria, as with some of those produced by LAB, are built from repeated oligosaccharide units, referred to here as “EPS-units.” The EPS-unit structure of a few bifido-EPS polymers has been elucidated by means of nuclear magnetic resonance (NMR). As could be expected from such chemical and structural complexity, there are several enzymes involved in EPS synthesis which are organized in *eps* clusters. The

use of genomic databases and bioinformatic tools allows the depiction of the genetic maps to describe potential biosynthetic pathways of EPS in bifidobacteria, such as the anabolic route of EPS production.

#### STRUCTURE AND GENETICS OF THE EPS-UNITS IN BIFIDOBACTERIA

In the 1990s, several articles reported the physicochemical characteristics of EPS synthesized by bifidobacteria belonging to the species *B. breve* and *B. longum*. In the second decade of this century, a renewed interest in this topic is noticeable as a consequence of the isolation of new EPS-producing strains from natural resources, such as the intestinal microbiota (37, 38). However, until now, only the structures of the EPS-units building the polymers produced by nine bifidobacterial strains have been elucidated by NMR techniques (Table 1). The monosaccharide composition of about 30 bifido-EPS has been analyzed by different chromatographic techniques (data are included in reference 26). This chemical characterization indicates that bifido-EPS are built with the same main monosaccharides—i.e., D-glucose, D-galactose, and L-rhamnose—as LAB-EPS. All bifido-EPS contain galactose, followed in abundance by glucose (28 of 30), and half of them also have rhamnose. In this regard, it has previously been indicated that rhamnose is found in a higher proportion in EPS synthesized by strains of human origin than in polymers isolated from strains of food origin (26). Besides, some particular EPS present a higher percentage of rhamnose in their composition. This is the case for the HMW-EPS fraction purified from strain *B. animalis* subsp. *lactis* IPLA-R1 (Table 1), whose EPS-unit contains 50% rhamnose (39). This fact has recently been correlated with an overexpression of genes coding for rhamnose biosynthesis precursors located inside the *eps* cluster of this strain (28). Therefore, taking into account the limitations imposed by the low number of bifido-EPS analyzed, we propose that they share common compositional traits with LAB-EPS, especially with those synthesized by strains of human origin.

With regard to the genetic analyses of EPS biosynthesis in members of the genus *Bifidobacterium*, reports in the literature are very scarce. The first defined *eps* cluster in bifidobacteria was that of strain *B. animalis* subsp. *lactis* IPLA-R1 (previously named A1dOxR). The cluster was delimited to a fragment of 54.3 kb, including 42 predicted genes flanked by a transposase-encoding gene. Based on homology studies, it was inferred that the genes present in this *eps* cluster were similar to those detected in LAB-*eps* clusters (39). Strain *B. breve* UCC20003 harbors an EPS-encoding cluster of 25.6 kb with 20 predicted genes, which are organized in two adjacent and oppositely oriented gene sets (8 genes in the *eps1* operon and 10 genes in the *eps2* operon). The other two genes for the priming glycosyltransferase (GTF) and the protein involved in chain length determination are transcribed by different promoters. It seems that the EPS synthesis in this strain could be bidirectional, thus producing one or two possible polymers (8).

The development of bioinformatic tools, as well as the increasing number of available genomes in public databases, has promoted the search for genes involved in the different metabolic pathways in bifidobacteria (49). In the next section, data about *eps* clusters that we identified from publicly available *Bifidobacterium* genomes are summarized. Our aim was to compare their functional-structural organization with that of a “consensus” LAB-*eps* cluster (24, 25, 48, 50).

TABLE 1 Repeating EPS-unit structures of polymers synthesized by *Bifidobacterium* determined with nuclear magnetic resonance<sup>a</sup>

Species	Strain	EPS-unit location	EPS-unit structure <sup>c</sup>	Reference
<i>B. animalis</i> subsp. <i>lactis</i>	IPLA-R1 (A1dOxR)	Extracellular	$\begin{array}{c} \alpha\text{-D-Galp}(1\rightarrow 2)\text{-}\alpha\text{-L-Rhap} \\ \downarrow 1 \\ 2 \\ \rightarrow 4\text{-}\beta\text{-D-Glcp}(1\rightarrow 3)\text{-}\alpha\text{-L-Rhap}(1\rightarrow 2)\text{-}\alpha\text{-L-Rhap}(1\rightarrow 4)\text{-}\beta\text{-D-Galp}(1\rightarrow \end{array}$	39
<i>B. animalis</i> subsp. <i>lactis</i>	RH	Cell wall	$\begin{array}{c} \alpha\text{-D-Galp} \qquad \qquad \qquad \alpha\text{-D-Galp} \\ \downarrow 1 \qquad \qquad \qquad \downarrow 1 \\ 3 \qquad \qquad \qquad 3 \\ \rightarrow 4\text{-}\alpha\text{-D-Glcp}(1\rightarrow 4)\text{-}\alpha\text{-D-Manf}(1\rightarrow 4)\text{-}\alpha\text{-L-Rha}(1\rightarrow 4)\text{-}\alpha\text{-D-Manf}(1\rightarrow 4)\text{-}\alpha\text{-D-Galp}(1 \rightarrow \end{array}$	40
<i>B. adolescentis</i>	YIT 4011	Cell wall	$\begin{array}{c} \beta\text{-Glc} \qquad \qquad \qquad \beta\text{-Glc} \qquad \beta\text{-Glc} \\ \downarrow 1 \qquad \qquad \qquad \downarrow 1 \qquad \downarrow 1 \\ 3 \qquad \qquad \qquad 3 \qquad 3 \\ \rightarrow 2\text{-}\alpha\text{-}6\text{dTal}(1\rightarrow 3)\text{-}\beta\text{-}6\text{dTal}(1\rightarrow 3)\text{-}\beta\text{-}6\text{dTal}(1\rightarrow 3)\text{-}\beta\text{-}6\text{dTal}(1\rightarrow 2)\text{-}\alpha\text{-}6\text{dTal}(1\rightarrow 2)\text{-}\alpha\text{-}6\text{dTal}(1\rightarrow \end{array}$	41
<i>B. bifidum</i>	BIM B-465	Cell wall	$\begin{array}{c} 6 \\ \uparrow \\ 1 \\ \alpha\text{-D-Glcp} \\ \rightarrow 3\text{-}\beta\text{-D-Glcp}(1\rightarrow 3)\text{-}\beta\text{-D-Galp}(1\rightarrow 4)\text{-}\alpha\text{-D-Galp}(1\rightarrow 2)\text{-}\alpha\text{-D-Glcp}(1 \rightarrow \end{array}$	42
<i>B. breve</i>	YIT 4010	Cell wall	$\begin{array}{c} 6 \\ \uparrow \\ 1 \\ \alpha\text{-D-Glcp} \\ \rightarrow 6\text{-}\beta\text{-D-Galp}(1\rightarrow 5)\text{-}\beta\text{-D-Galp}(1\rightarrow \end{array}$	43
<i>B. catenulatum</i>	YIT 4016	Cell wall	$\begin{array}{c} 6 \\ \uparrow \\ 1 \\ \alpha\text{-D-Galp} \\ \rightarrow 3\text{-}\beta\text{-D-Galp}(1\rightarrow 3)\text{-}\alpha\text{-D-Galp}(1\rightarrow \end{array}$	44
<i>B. infantis</i>	ATCC 15697	Cell wall <sup>b</sup>	$\begin{array}{c} 6 \\ \uparrow \\ 1 \\ \alpha\text{-D-Galp} \\ \rightarrow 2\text{-}\alpha\text{-L-Rhap}(1\rightarrow 3)\text{-}\alpha\text{-D-Galp}(1\rightarrow 2)\text{-}\alpha\text{-L-Rhap}(1\rightarrow 3)\text{-}\alpha\text{-D-Galp}(1\rightarrow \end{array}$	45
<i>B. longum</i>	YIT 4028	Cell wall	$\begin{array}{c} 6 \\ \uparrow \\ 1 \\ \alpha\text{-D-Galp} \\ \rightarrow 4\text{-}\alpha\text{-D-Galp}(1\rightarrow 4)\text{-}\alpha\text{-D-Galp}(1\rightarrow 4)\text{-}\beta\text{-D-Glcp}(1\rightarrow 3)\text{-}\alpha\text{-D-Galp}(1\rightarrow 3)\text{-}\beta\text{-L-Rhap}(1\rightarrow \end{array}$	46
<i>B. longum</i>	JBL05	Extracellular	$\begin{array}{c} \beta\text{-D-Glcp} \\ \downarrow 1 \\ 6 \\ \rightarrow 4\text{-}\alpha\text{-D-Galp}(1\rightarrow 4)\text{-}\alpha\text{-D-Galp}(1\rightarrow 4)\text{-}\beta\text{-D-Glcp}(1\rightarrow 3)\text{-}\alpha\text{-D-Galp}(1\rightarrow 3)\text{-}\beta\text{-L-Rhap}(1\rightarrow \end{array}$	47

<sup>a</sup> Data have been updated from reference 48.

<sup>b</sup> This backbone is partially substituted with  $\beta$ -D-Glcp linked in the carbon C-6 of both monosaccharides.

<sup>c</sup> Glc, glucose; Gal, galactose; Rha, rhamnose; Mn, mannose; 6dTal, 6-deoxy-talose; p, pyranose ring conformation; f, furanose ring conformation.

### IN SILICO ANALYSIS OF BIFIDO-eps CLUSTERS

The completed genomes of 28 bifidobacteria, held in the GenBank database at the time of writing, were used to search for putative bifido-eps clusters (see Table S1 in the supplemental material). The genome of *B. longum* subsp. *longum* NCC2705, which was the first to become publicly available (51), was used to search for the

priming-GTF gene (*p-gtf*). This is the enzyme that catalyzes the initial step of the EPS-unit synthesis; therefore, it should be present in all *eps* clusters. Strain NCC2705 harbors two putative *p-gtf* genes annotated as “undecaprenyl-phosphate sugar phosphotransferase” (*rfbP*) and “galactosyl-transferase” (*cpsD*), coding for proteins of 573 amino acids (accession number NP\_695455) and

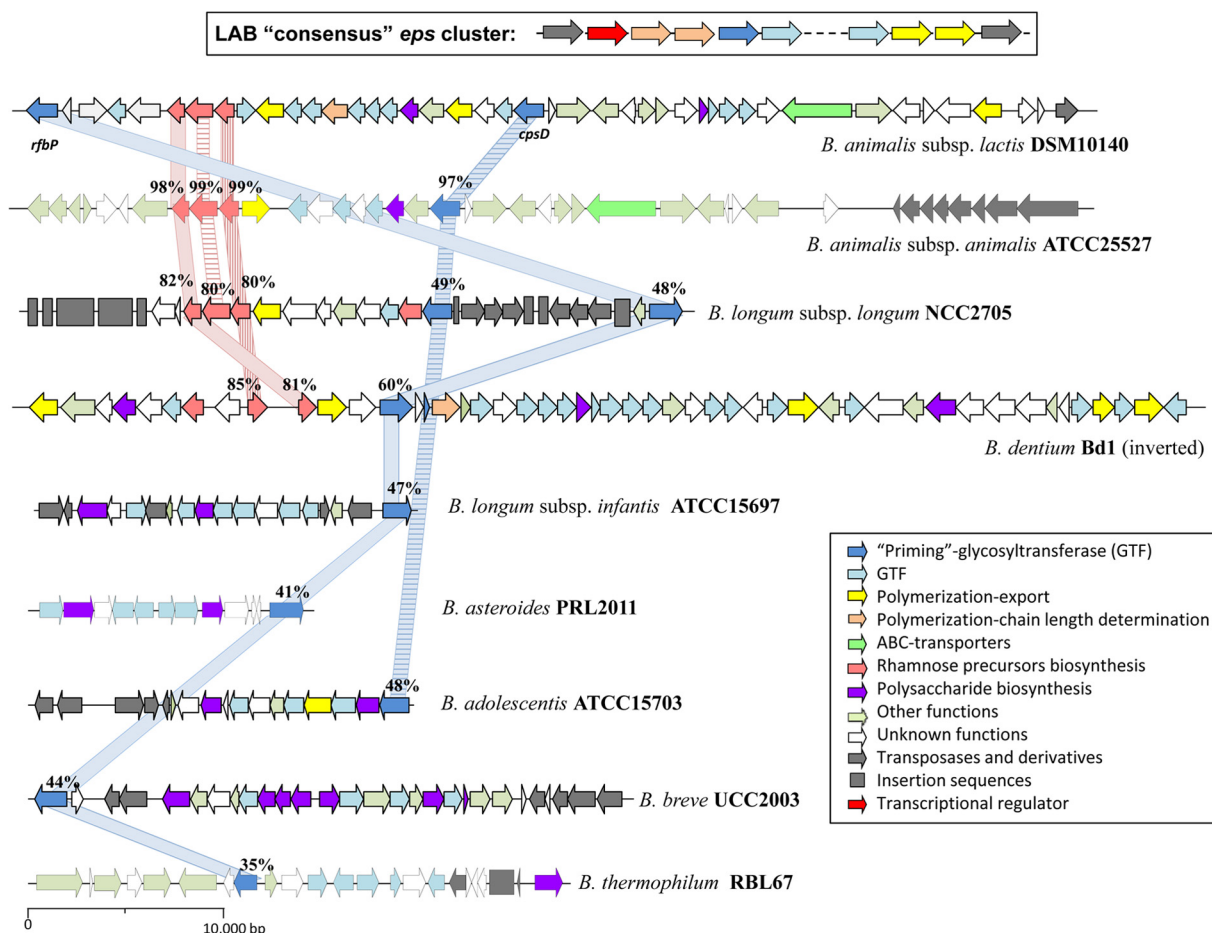


FIG 2 Physical maps of the putative *eps* clusters from nine representative *Bifidobacterium* species. A “consensus” *eps* cluster for LAB (based on reviews in references 23, 24, 25, and 48) is also depicted. The genes were categorized according to their potential functions, which are indicated with the colored arrows in the box. For each strain, the amino acid identity (percentage) of the priming GTF (light blue line) and the rhamnose biosynthesis (light red line) with respect to those of *B. animalis* subsp. *lactis* DSM 10140 is indicated.

514 amino acids (accession number NP\_695447), respectively. The percentage of amino acid homology between these two priming-GTFs was 38%. Using *rfbP* and *cpsD* genes as the templates, the other available bifidobacterial genome sequences were screened for the presence of *p-gtf*. Most of them seemed to harbor at least one *p-gtf* (see Table S1), except *B. bifidum* PRL2010 and BGN4 and *B. longum* subsp. *longum* DJ010A and JDM301. Following the identification of the gene(s) encoding the priming-GTF, the regions surrounding these genes were searched for those encoding other proteins described as being implicated in the synthesis of LAB-EPS (Fig. 2). All strains, except *B. bifidum* S17 and *B. longum* subsp. *infantis* 157F, presented several of those genes closely located to *p-gtf*, thus having an *eps* cluster-like structure (see Table S1). Indeed, the putative *eps* cluster of strain *B. animalis* subsp. *lactis* DSM 10140 (GenBank accession no. CP001606) showed an operon-like structure containing most of the genes described in the “consensus” LAB-*eps* cluster. Thus, the defined *eps* cluster from DSM 10140 was used as a template to perform a local BLAST comparison with the rest of the completed bifidobacterial genomes. From the 22 bifidobacterial genomes having an *eps* cluster, one representative of each species/subspecies was chosen to depict physical maps showing the putative functions of the proteins linked to the cluster (Fig. 2).

The first noticeable outcome from the *in silico* analysis of the bifido-*eps* clusters is the lack of a “consensus” functional-structural organization such as that described for LAB genera among different *Bifidobacterium* species. There is a consistent interspecies variability in the bifido-*eps* cluster length (see Table S1 in the supplemental material), as well as in the numbers and types of gene present (Fig. 2). The variability found in the genetic content responsible for the bifido-EPS anabolic route could reflect the genome plasticity reported in the *Bifidobacterium* genus (52). Regarding intraspecies diversity, only genomes from *B. longum* subsp. *longum* and *B. animalis* subsp. *lactis* have been sequenced in higher numbers allowing a tentative comparison. In the case of *B. longum* subsp. *longum*, the six strains that harbor *eps* genes presented a different structural organization (see Fig. S1 in the supplemental material). In this regard, Lukjancenko et al. (53) have shown that the percentage of homology among total proteomes derived from five *B. longum* genomes is lower than 69%. In contrast, the degree of similarity obtained for six *B. animalis* subsp. *lactis* strains was higher (from 82.5% to 99.5%). A low degree of interstrain variability was reported for the latter subspecies using comparative genomics (54). Our results, obtained with the bifido-*eps* clusters analyzed from nine *B. animalis* subsp. *lactis* strains, supported this finding; they had similar lengths (see Table S1) and identical genetic structures, presenting only a few single nucleotide polymorphisms

(55). Although the number of genomes analyzed is not large enough to obtain any evolutionary conclusion, we are tempted to hypothesize that in LAB-*eps* clusters a conserved functional structure could have been present in a common ancestor before LAB-genus divergence. However, the acquisition of *eps* clusters in *Bifidobacterium* could have been a more recent event.

In spite of the lack of a “consensus” structural organization in bifido-*eps* clusters, most genes described in LAB-*eps* have been detected in the *Bifidobacterium* genomes screened, for example, a variable number of genes coding for different GTFs. In general, the degree of homology in the amino acid sequences of the GTFs from the different strains tested was very low, and that of *B. dentium* was the lowest (data not shown). In contrast, the interspecies homology of the priming-GTF was high and was even higher than that detected with the priming-GTF harbored by the same strain (Fig. 2). The high homology detected for each priming-GTF among the species could be due to the presence of conserved domains involved in the interaction with the lipid carrier (37, 56). The divergences between the two RfbP and CpsD priming-GTFs could be due to domains related to the sugar specificity of each enzyme.

Two types of genes that could be involved in the transport of the repeated units across the cytoplasmic membrane were detected in bifido-*eps*: “flippase”-like and ABC-transporter genes (Fig. 2). The genes for the flippase-like transport and polymerization system, which is also present in LAB-*eps*, were detected in most of the bifido-*eps* clusters analyzed. However, ABC-transporter genes are less abundant and were not described in LAB-*eps*. Another feature common between LAB-*eps* and bifido-*eps* clusters is the presence of genes encoding transposases and insertion sequences (Fig. 2). The presence of such elements could be related to the putative horizontal transfer of *eps* genes among different taxa. A remarkable difference detected in bifido-*eps* clusters with respect to those of LAB is the absence of (known) transcriptional regulator genes in the neighborhood of the cluster. These are currently found at the 5′ end in *eps* clusters of most LAB species. Some of the genes with unknown functions located inside or close to the bifido-*eps* cluster could encode proteins that regulate the EPS synthesis. It is also possible that these regulator genes are located in another region of the bifidobacterial genome. Finally, several genes coding for precursors involved in the biosynthesis of rhamnose were found within the *eps* cluster of *B. animalis* subsp. *lactis* DSM 10140, *B. animalis* subsp. *animalis* ATCC 25527, *B. longum* subsp. *longum* NCC2705, and *B. dentium* Bd1 (Fig. 2). These genes coding for three proteins, dTDP-glucose pyrophosphorylase, dTDP-4-dehydrorhamnose 3,5-epimerase, and dTDP-D-glucose 4,6-dehydratase, show high amino acid homology among the species (currently higher than 80%) (Fig. 2). In addition, the G+C content of rhamnose-precursor genes is higher than that of the *eps* cluster, which suggests that these genes were not acquired by horizontal transfer; thus, they could be involved in the metabolism of this sugar in a manner separate from their role in the synthesis of EPS.

#### HYPOTHETICAL PATHWAY OF EPS SYNTHESIS IN *B. ANIMALIS* SUBSP. *LACTIS*

The mechanism of EPS biosynthesis in the genus *Bifidobacterium* is not known, and the biosynthetic pathway in LAB has been proposed on the basis of the functional analysis of a few genes and, mainly, on the basis of sequence homology studies (23–25, 48). The composition and the structure of bifido-EPS have not been

characterized for any of the strains whose genomes have been completed. Thus, a correlation between the genetics and physico-chemical characteristics of the bifido-EPS cannot be established. However, the EPS-unit of the HMW-EPS synthesized by strain *B. animalis* subsp. *lactis* IPLA R1, as well as the sequence of its *eps* cluster, is known (39). Therefore, taking into account the predicted function assigned to some genes in this bifido-*eps* cluster, we propose a hypothetical pathway for the synthesis of the HMW-EPS in this strain (Fig. 3). It is worth noting that this route of synthesis could be extended to any species of *Bifidobacterium*. The synthesis of most heteropolysaccharides requires the use of activated sugar nucleotide (nucleoside di- or monophosphorylated sugar) precursors which are formed from the intermediate molecules of glycolysis (18). The first step in the formation of the EPS-unit would be catalyzed by the priming-GTF; in the case of *B. animalis* subsp. *lactis*, the two *p-gtf* genes seem to be functional (13, 28). This enzyme would transfer a sugar-1-phosphate (not identified in EPS IPLA-R1) to the lipophilic carrier molecule anchored to the cell membrane. However, priming-GTFs are not able to catalyze the formation of glycosidic bonds. These linkages are made by other GTFs, which transfer new sugar moieties from the donor nucleotide sugars to the initial monosaccharide of the unit. Thus, depending on the structure of each EPS-unit, the numbers and specificities of GTF differ; in the case of the IPLA-R1 polymer, enzymes catalyzing the transfer of glucose, galactose, and rhamnose are required. Once the EPS-unit is formed in the cytoplasm, an export-polymerization process is necessary to move the finished unit to the extracellular face of the cytoplasmic membrane and to build the polymer. The presence of flippase-like genes in most bifido-*eps* clusters (Fig. 2) suggests that a system dependent on this enzyme (57) is active in bifidobacteria. Most of the information related to this export system has been obtained from studies of the O-antigen LPS synthesis in *Escherichia coli* (58). The flippase-like protein (named Wzx in *E. coli*) translocates the lipophilic-carrier linked EPS-unit across the cell membrane. Following this translocation event, a polymerase (Wzy) assembles the repeating units. Finally, proteins responsible for chain-length determination (Wzz) are involved in terminating chain elongation and thus influence EPS molecular mass.

#### CONCLUDING REMARKS

The *in silico* analysis performed with the *Bifidobacterium* genomes available in public databases allowed us to conclude that there is not a “consensus” structural organization of the *eps* clusters corresponding to that detected in LAB-*eps* clusters. Nevertheless, some common features of bifido-*eps* clusters can be proposed: (i) high inter- and intraspecies organizational variability, with the exception of *B. animalis* subsp. *lactis*, (ii) lower G+C content of the *eps* cluster, in comparison with that of the total bifidobacterial genome, (iii) in some cases, association with genes involved in the synthesis of rhamnose, (iv) presence of genes encoding a “flippase”-like export-polymerization system that could be the mechanism active in bifido-EPS synthesis, and (v) apparent lack of transcriptional regulator genes. The increased availability of genomes will allow us, in the near future, to confirm, discard, or extend the characteristics detected until now in bifido-*eps* clusters. Additionally, in order to gain further insight into the EPS biosynthesis in the *Bifidobacterium* genus, more studies connecting genetic information with the physicochemical characteristics of the EPS are required. This is a vital step to be able to understand the

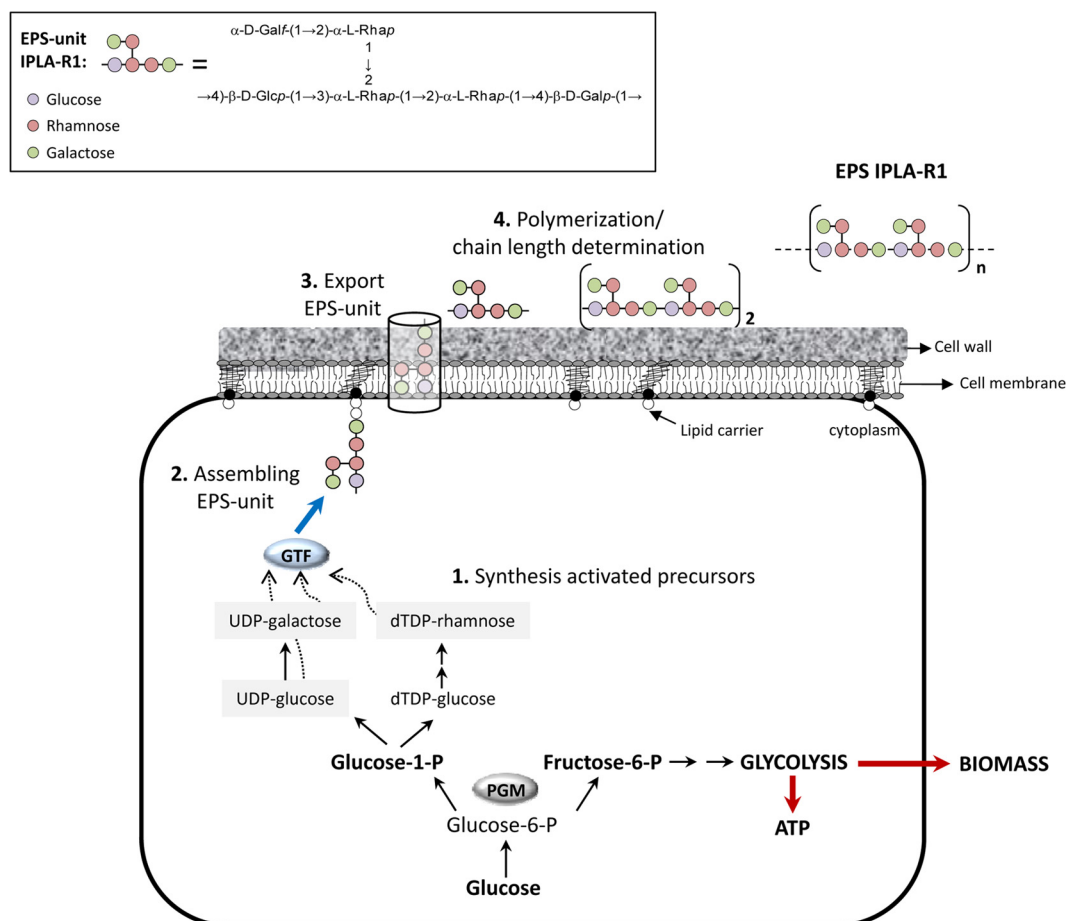


FIG 3 Hypothetical EPS biosynthesis pathway in the strain *Bifidobacterium animalis* subsp. *lactis* IPLA-R1 based on the known structure of its high-molecular-weight EPS unit (39) and on the homology analysis of its *eps* cluster (see Fig. 2). GTF, glycosyltransferase; PMG, phosphoglucomutase.

biological properties for either the producing bacteria or the host. This exciting topic constitutes an interesting opportunity for future research in the probiotic field.

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## REFERENCES

1. Rehm BH. 2010. Bacterial polymers: biosynthesis, modifications and applications. *Nat. Rev. Microbiol.* 8:578–592. <http://dx.doi.org/10.1038/nrmicro2354>.
2. Freitas F, Alves VD, Reis MAM. 2011. Advances in bacterial exopolysaccharides: from production to biotechnological applications. *Trends Biotechnol.* 29:388–398. <http://dx.doi.org/10.1016/j.tibtech.2011.03.008>.
3. Koo H, Xiao J, Klein MI, Jeon JG. 2010. Exopolysaccharides produced by *Streptococcus mutans* glycosyltransferases modulate the establishment of microcolonies within multispecies biofilms. *J. Bacteriol.* 192:3024–3032. <http://dx.doi.org/10.1128/JB.01649-09>.
4. Gonzalez-Escobedo G, Marshall JM, Gunn JS. 2011. Chronic and acute infection of the gall bladder by *Salmonella* Thypi: understanding the carrier state. *Nat. Rev. Microbiol.* 9:9–14. <http://dx.doi.org/10.1038/nrmicro2490>.
5. Round JL, Mazmanian SK. 2009. The gut microbiota shapes intestinal immune responses during health and disease. *Nat. Rev. Microbiol.* 9:313–323. <http://dx.doi.org/10.1038/nri2515>.
6. Round JL, Lee SM, Li J, Tran G, Jabri B, Chatila TA, Mazmanian SK. 2011. The Toll-like receptor 2 pathway established colonization by a commensal of the human microbiota. *Science* 322:974–977. <http://dx.doi.org/10.1126/science.1206095>.
7. Lebeer S, Claes IJJ, Verhoeven TLA, Vanderleyden J, De Keersmaecker SC. 2011. Exopolysaccharides of *Lactobacillus rhamnosus* GG form a protective shield against innate immune factors in the intestine. *Microb. Biotechnol.* 4:368–374. <http://dx.doi.org/10.1111/j.1751-7915.2010.00199.x>.
8. Fanning S, Hall LJ, Cronin M, Zomer A, MacSharry J, Goulding D, O’Connell-Motherway M, Shanahan F, Nally K, Dougan G, van Sinderen D. 2012. Bifidobacterial surface-exopolysaccharide facilitates commensal-host interaction through immune modulation and pathogen protection. *Proc. Natl. Acad. Sci. U. S. A.* 109:2108–2113. <http://dx.doi.org/10.1073/pnas.1115621109>.
9. López P, Monteserín DC, Gueimonde M, de los Reyes-Gavilán CG, Margolles A, Suárez A, Ruas-Madiedo P. 2012. Exopolysaccharide-producing *Bifidobacterium* strains elicit different *in vitro* responses upon interaction with human cells. *Food Res. Int.* 46:99–107. <http://dx.doi.org/10.1016/j.foodres.2011.11.020>.
10. Nikolic M, López P, Strahinic I, Suárez A, Kojic M, Fernández-García M, Topisirovic L, Golic N, Ruas-Madiedo P. 2012. Characterization 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. <http://dx.doi.org/10.1016/j.ijfoodmicro.2012.07.015>.
11. Denou E, Pridmore RD, Berger B, Panoff JM, Arigoni F, Brüssow H.

2008. Identification of genes associated with the long-gut-persistence phenotype of the probiotic *Lactobacillus johnsonii* strain NCC522 using a combination of genomic and transcriptomic analysis. *J. Bacteriol.* 190: 3161–3168. <http://dx.doi.org/10.1128/JB.01637-07>.
12. Lebeer S, Verhoeven TLA, Perea-Velez M, Vanderleyden J, De Keersmaecker SCJ. 2007. Impact of environmental and genetic factors on biofilm formation by probiotic strains *Lactobacillus rhamnosus* GG. *Appl. Environ. Microbiol.* 73:6768–6775. <http://dx.doi.org/10.1128/AEM.01393-07>.
  13. Ruas-Madiedo P, Gueimonde M, Arigoni F, de los Reyes-Gavilán CG, Margolles A. 2009. Bile affects the synthesis of exopolysaccharides by *Bifidobacterium animalis*. *Appl. Environ. Microbiol.* 75:1204–1207. <http://dx.doi.org/10.1128/AEM.00908-08>.
  14. 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). [http://www.fao.org/icalog/search/dett.asp?aries\\_id=107387](http://www.fao.org/icalog/search/dett.asp?aries_id=107387).
  15. Ventura M, Turroni F, O'Connell-Motherway M, MacSharry J, van Sinderen D. 2012. Host-microbe interactions that facilitate gut colonization by commensal bifidobacteria. *Trends Microbiol.* 20:467–476. <http://dx.doi.org/10.1016/j.tim.2012.07.002>.
  16. Turroni F, Ventura M, Buttó LF, Duranti S, O'Toole PW, O'Connell-Motherway M, van Sinderen D. 2013. Molecular dialogue between the human gut microbiota and the host: a *Lactobacillus* and *Bifidobacterium* perspective. *Cell. Mol. Life Sci.* [Epub ahead of print.] <http://dx.doi.org/10.1007/s0018-013-1318-0>.
  17. Cronin M, Ventura M, Fitzgerald GF, van Sinderen D. 2011. Progress in genomics, metabolism and biotechnology of bifidobacteria. *Int. J. Food Microbiol.* 149:4–18. <http://dx.doi.org/10.1016/j.ijfoodmicro.2011.01.019>.
  18. De Vuyst L, De Vin F, Vaningelgem F, Degeest B. 2001. Recent developments in the biosynthesis and applications of heteropolysaccharides from lactic acid bacteria. *Int. Dairy J.* 11:687–707. [http://dx.doi.org/10.1016/S0958-6946\(01\)00114-5](http://dx.doi.org/10.1016/S0958-6946(01)00114-5).
  19. Behare PV, Singh R, Kumar M, Prajapati JB, Singh RP. 2009. Exopolysaccharides of lactic acid bacteria: a review. *J. Food Sci. Technol.* 46:1–11.
  20. Badel S, Bernardi T, Michaud P. 2011. New perspectives for lactobacilli exopolysaccharides. *BioTechnol. Adv.* 29:54–66. <http://dx.doi.org/10.1016/j.biotechadv.2010.08.011>.
  21. Hassan AN. 2008. Possibilities and challenges of exopolysaccharide-producing lactic cultures in dairy foods. *J. Dairy Sci.* 91:1282–1298. <http://dx.doi.org/10.3168/jds.2007-0558>.
  22. Ruas-Madiedo P, Salazar N, de los Reyes-Gavilán CG. 2009. Exopolysaccharides produced by lactic acid bacteria in food and probiotic applications, p 887–902. In Moran A, Holst O, Brennan PJ, von Itzstein M (ed), *Microbial glycobiology. Structures, relevance and applications*. Academic Press, Elsevier, London, United Kingdom.
  23. Boels IC, van Kranenburg R, Hugenholtz J, Kleerebezem M, de Vos WM. 2001. Sugar catabolism and its impact on the biosynthesis and engineering of exopolysaccharide production in lactic acid bacteria. *Int. Dairy J.* 11:723–732. [http://dx.doi.org/10.1016/S0958-6946\(01\)00116-9](http://dx.doi.org/10.1016/S0958-6946(01)00116-9).
  24. Jolly L, Stingle F. 2001. Molecular organization and functionality of exopolysaccharide gene clusters in lactic acid bacteria. *Int. Dairy J.* 11:733–745. [http://dx.doi.org/10.1016/S0958-6946\(01\)00117-0](http://dx.doi.org/10.1016/S0958-6946(01)00117-0).
  25. Broadbent JR, McMahon DJ, Welker DL, Oberg CJ, Moineau S. 2003. Biochemistry, genetics, and applications of exopolysaccharide production in *Streptococcus thermophilus*: a review. *J. Dairy Sci.* 86:407–423. [http://dx.doi.org/10.3168/jds.S0022-0302\(03\)73619-4](http://dx.doi.org/10.3168/jds.S0022-0302(03)73619-4).
  26. Hidalgo-Cantabrana C, López P, Gueimonde M, de los Reyes-Gavilán CG, Suárez A, Margolles A, Ruas-Madiedo P. 2012. Immune modulation capability of exopolysaccharides synthesised by lactic acid bacteria and bifidobacteria. *Proteins Antimicrob. Proteins* 4:227–237. <http://dx.doi.org/10.1007/s12602-012-9110-2>.
  27. Salazar N, Gueimonde M, de los Reyes-Gavilán CG, Ruas-Madiedo P. Exopolysaccharide produced by lactic acid bacteria and bifidobacteria as fermentable substrates by the intestinal microbiota. *Crit. Rev. Food Sci. Nutr.*, in press. <http://dx.doi.org/10.1080/10408398.2013.770728>.
  28. Hidalgo-Cantabrana C, Sánchez B, Moine D, Berger B, de los Reyes-Gavilán CG, Gueimonde M, Margolles A, Ruas-Madiedo P. 2013. Insights into the rosy phenotype of the exopolysaccharide-producing strain *Bifidobacterium animalis* subsp. *lactis* A1dOxR. *Appl. Environ. Microbiol.* 79:3870–3874. <http://dx.doi.org/10.1128/AEM.00633-13>.
  29. Wu MH, Pan TM, Wu YJ, Chang SJ, Chang MS, Hu CY. 2010. Exopolysaccharide activities from probiotic *Bifidobacterium*: immunomodulatory effects (on J774A. 1 macrophages) and antimicrobial properties. *Int. J. Food Microbiol.* 144:104–110. <http://dx.doi.org/10.1016/j.ijfoodmicro.2010.09.003>.
  30. Ruas-Madiedo P, Gueimonde M, Margolles A, de los Reyes-Gavilán CG, Salminen S. 2006. Exopolysaccharides produced by probiotic strains modify the adhesion of probiotics and enteropathogens to human intestinal mucus. *J. Food Prot.* 69:2011–2015.
  31. Ruas-Madiedo P, Medrano M, Salazar N, de los Reyes-Gavilán CG, Pérez PF, Abraham AG. 2010. Exopolysaccharides produced by *Lactobacillus* and *Bifidobacterium* strains abrogate in vitro the cytotoxic effect of bacterial toxins on eukaryotic cells. *J. Appl. Microbiol.* 109:2079–2086. <http://dx.doi.org/10.1111/j.1365-2672.2010.04839.x>.
  32. Xu R, Shang N, Li P. 2011. *In vitro* and *in vivo* antioxidant activity of exopolysaccharide fractions from *Bifidobacterium animalis* RH. *Anaerobe* 17:226–231. <http://dx.doi.org/10.1016/j.anaerobe.2011.07.010>.
  33. Lebeer S, Vanderleyden J, De Keersmaecker SCJ. 2010. Host interactions of probiotic bacterial surface molecules: comparison with commensals and pathogens. *Nat. Rev. Microbiol.* 8:171–184. <http://dx.doi.org/10.1038/nrmicro2297>.
  34. Bron PA, van Baaren P, Kleerebezem M. 2012. Emerging molecular insights into the interaction between probiotics and the host intestinal mucosa. *Nat. Rev. Microbiol.* 10:66–78. <http://dx.doi.org/10.1038/nrmicro2690>.
  35. Salazar N, Gueimonde M, Hernández-Barranco AM, Ruas-Madiedo P, de los Reyes-Gavilán CG. 2008. Exopolysaccharide produced by intestinal *Bifidobacterium* strains act as fermentable substrates for human intestinal bacteria. *Appl. Environ. Microbiol.* 74:4737–4745. <http://dx.doi.org/10.1128/AEM.00325-08>.
  36. Salazar N, Ruas-Madiedo P, Kolida S, Collins M, Rastall R, Gibson G, de los Reyes-Gavilán CG. 2009. 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. <http://dx.doi.org/10.1016/j.ijfoodmicro.2009.08.017>.
  37. Ruas-Madiedo P, Moreno JA, Salazar N, Delgado S, Mayo B, Margolles A, de los Reyes-Gavilán CG. 2007. Screening of exopolysaccharide-producing *Lactobacillus* and *Bifidobacterium* strains isolated from the human intestinal microbiota. *Appl. Environ. Microbiol.* 73:4385–4388. <http://dx.doi.org/10.1128/AEM.02470-06>.
  38. Prasanna PHP, Grandison AS, Charalampopoulos D. 2012. Screening human intestinal *Bifidobacterium* strains for growth, acidification, EPS production and viscosity potential in low-fat milk. *Int. Dairy J.* 23:36–44. <http://dx.doi.org/10.1016/j.idairyj.2011.09.008>.
  39. Leivers S, Hidalgo-Cantabrana C, Robinson G, Margolles A, Ruas-Madiedo P, Laws AP. 2011. Structure of the high molecular weight exopolysaccharide produced by *Bifidobacterium animalis* subsp. *lactis* IPLA-R1 and sequence analysis of its putative *eps* cluster. *Carbohydr. Res.* 346:2710–2717. <http://dx.doi.org/10.1016/j.carres.2011.09.010>.
  40. Shang N, Xu R, Li P. 2013. Structure characterization of an exopolysaccharide produced by *Bifidobacterium animalis* RH. *Carbohydr. Polym.* 91:128–134. <http://dx.doi.org/10.1016/j.carbpol.2012.08.012>.
  41. Nagaoka M, Muto M, Yokokura T, Mutai M. 1988. Structure of 6-deoxytalose-containing polysaccharide from the cell wall of *Bifidobacterium adolescentis*. *J. Biochem.* 103:618–621.
  42. Zdorovenko EL, Kachala VV, Sidarenka AV, Izhik AV, Kisileva EP, Shashkov AS, Novik GI, Knirel YA. 2009. Structure of the cell wall polysaccharides of probiotic bifidobacteria *Bifidobacterium bifidum* BIM M-465. *Carbohydr. Res.* 344:2417–2420. <http://dx.doi.org/10.1016/j.carres.2009.08.039>.
  43. Habu Y, Nagaoka M, Yokokura T, Azuma I. 1987. Structural studies of cell wall polysaccharides from *Bifidobacterium breve* YIT 4010 and related *Bifidobacterium* species. *J. Biochem.* 102:1423–1432.
  44. Nagaoka M, Hahimoto S, Shibata H, Kimura I, Kimura K, Sawada H, Yokokura T. 1996. Structure of a galactan from cell walls of *Bifidobacterium catenulatum* YIT4016. *Carbohydr. Res.* 281:285–291. [http://dx.doi.org/10.1016/0008-6215\(95\)00354-1](http://dx.doi.org/10.1016/0008-6215(95)00354-1).
  45. Tone-Shimokawa Y, Toida T, Kawashima T. 1996. Isolation and structural analysis of polysaccharide containing galactofuranose from the walls of *Bifidobacterium infantis*. *J. Bacteriol.* 178:317–320.
  46. Nagaoka M, Shibata H, Kimura I, Hashimoto S, Kimura K, Sawada H, Yokokura T. 1995. Structural studies on a cell wall polysaccharide from *Bifidobacterium longum* YIT4028. *Carbohydr. Res.* 274:245–249. [http://dx.doi.org/10.1016/0008-6215\(95\)00076-6](http://dx.doi.org/10.1016/0008-6215(95)00076-6).
  47. Kohno M, Suzuki S, Kanaya T, Yoshino T, Matsuura Y, Asada M,



- Kitamura S. 2009. Structural characterization of the extracellular polysaccharide produced by *Bifidobacterium longum* JBL05. *Carbohydr. Polym.* 77:351–357. <http://dx.doi.org/10.1016/j.carbpol.2009.01.013>.
48. Ruas-Madiedo P, Salazar N, de los Reyes-Gavilán CG. 2009. Biosynthesis and chemical composition of exopolysaccharides produced by lactic acid bacteria, p 279–310. *In* Ullrich M (ed), *Bacterial polysaccharides. Current innovations and future trends*. Caister Academic Press, Norfolk, United Kingdom.
49. Lee JH, O'Sullivan DJ. 2010. Genomic insights into bifidobacteria. *Microbiol. Mol. Biol. Rev.* 74:378–416. <http://dx.doi.org/10.1128/MMBR.00004-10>.
50. Berger B, Pridmore RD, Barretto C, Delmas-Julien F, Schreiber K, Arigoni F, Brüßow H. 2007. Similarity and differences in the *Lactobacillus acidophilus* group identified by polyphasic analysis and comparative genomics. *J. Bacteriol.* 189:1311–1321. <http://dx.doi.org/10.1128/JB.01393-06>.
51. Schell MA, Karmirantzou M, Snel B, Vilanova D, Berger B, Pessi G, Zwahlen MC, Desiere F, Bork P, Delley M, Pridmore D, Arigoni F. 2002. The genome sequence of *Bifidobacterium longum* reflects its adaptation to the human gastrointestinal tract. *Proc. Natl. Acad. Sci. U. S. A.* 99:14422–14427. <http://dx.doi.org/10.1073/pnas.212527599>.
52. Bottacini F, Medini D, Pavesi A, Turrone F, Forini E, Riley D, Giubellini V, Tattelin H, van Sinderen D, Ventura M. 2010. Comparative genomics of genus *Bifidobacterium*. *Microbiology* 156:3243–3254. <http://dx.doi.org/10.1099/mic.0.039545-0>.
53. Lukjancenko O, Ussery DW, Wassenaar TM. 2012. Comparative genomics of *Bifidobacterium*, *Lactobacillus* and related probiotic genera. *Microb. Ecol.* 63:651–673. <http://dx.doi.org/10.1007/s00248-011-9948-y>.
54. Milani C, Duranti S, Lugli GA, Bottacini F, Strati F, Arioli S, Foroni E, Turrone F, van Sinderen D, Ventura M. 2013. Comparative genomics of *Bifidobacterium animalis* subsp. *lactis* reveals a strict monophyletic bifidobacteria taxon. *Appl. Environ. Microbiol.* 79:4304–4315. <http://dx.doi.org/10.1128/AEM.00984-13>.
55. Ruas-Madiedo P, Sánchez B, Hidalgo-Cantabrana C, Margolles A, Laws A. 2012. Exopolysaccharides from lactic acid bacteria and bifidobacteria, p 125–151. *In* Hui YH, Evranuz EO (ed), *Handbook of animal-based fermented food and beverage technology*. CRC Press, Taylor and Francis Group, London, United Kingdom.
56. Provencher C, LaPointe G, Sirois S, van Calsteren MR, Roy D. 2003. Consensus-degenerate hybrid oligonucleotide primers for amplification of priming glycosyltransferase genes of the exopolysaccharide locus in strains of *Lactobacillus casei* group. *Appl. Environ. Microbiol.* 69:3299–3307. <http://dx.doi.org/10.1128/AEM.69.6.3299-3307.2003>.
57. Reid AN, Szymanski CM. 2009. Biosynthesis and assembly of capsular polysaccharides, p 351–373. *In* Moran A, Holst O, Brennan PJ, von Itzstein M (ed), *Microbial glycobiology. Structures, relevance and applications*. Academic Press, Elsevier, London, United Kingdom.
58. Reeves PR, Cunneen MM. 2009. Biosynthesis of O-antigen chains and assembly, p 319–335. *In* Moran A, Holst O, Brennan PJ, von Itzstein M (ed), *Microbial glycobiology. Structures, relevance and applications*. Academic Press, Elsevier, London, United Kingdom.

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