Genetic Mechanisms of Prebiotic Oligosaccharide Metabolism in Probiotic Microbes

Yong Jun Goh* and Todd R. Klaenhammer

Department of Food, Bioprocessing and Nutrition Sciences, North Carolina State University, Raleigh, North Carolina 27695; email: yjgoh@ncsu.edu, klaenhammer@ncsu.edu

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*Corresponding author

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Abstract

Recent insights into the relationship between the human gut and its resident microbiota have revolutionized our appreciation of this symbiosis and its impact on health and disease development. Accumulating evidence on probiotic and prebiotic interventions has demonstrated promising effects on promoting gastrointestinal health by modulating the microbiota toward the enrichment of beneficial microorganisms. However, the precise mechanisms of how prebiotic nondigestible oligosaccharides are metabolized by these beneficial microbes in vivo remain largely unknown. Genome sequencing of probiotic lactobacilli and bifidobacteria has revealed versatile carbohydrate metabolic gene repertoires dedicated to the catabolism of various oligosaccharides. In this review, we highlight recent findings on the genetic mechanisms involved in the utilization of prebiotic fructooligosaccharides, β-galactooligosaccharides, human milk oligosaccharides, and other prebiotic candidates by these probiotic microbes.

1. INTRODUCTION

Our gastrointestinal tract (GIT) represents one of the most densely populated ecological niches in nature, hosting an enormously complex and dynamic community of microbes (Xu et al. 2007). Through adaptive coevolution with humans, these trillions of microorganisms, known as the gut microbiota, collectively function as an organ that complements the human metabolic and immune systems (Backhed et al. 2007). Perturbation of the diversity of this microbial community has profound effects on host health, both intestinal and extraintestinal, and has been linked to diseases such as inflammatory bowel disease, irritable bowel syndrome, celiac disease, diabetes, cardiovascular disease, and brain-gut axis disorders (Bustos Fernandez et al. 2014, Goldsmith & Sartor 2014, Grenham et al. 2011, Petschow et al. 2013, Tilg & Moschen 2014). Although the human diet is generally rich in complex carbohydrates, the human genome encodes fewer than 20 glycosidases capable of hydrolyzing only sucrose, lactose, and, to some extent, starch (Cantarel et al. 2012). Thus, most of these indigestible complex carbohydrates reach the intestine intact and are metabolized by select members of the gut microbiota able to transport and metabolize these compounds. The concept of prebiotic was introduced in the 1990s with the aim to selectively enrich beneficial commensal and probiotic gut microbes, specifically *Lactobacillus* and *Bifidobacterium* (Gibson & Roberfroid 1995, Gibson et al. 2010). The currently established prebiotic ingredients are fructooligosaccharides (FOSs) and β-galactooligosaccharides (GOSs) (Blatchford et al. 2013). Selective stimulation of bifidobacteria and lactobacilli by these nondigestible oligosaccharides has been well documented both in vitro and in vivo (Davis et al. 2011, Gibson et al. 1995, Moro et al. 2002). Several emerging prebiotic candidates such as glucooligosaccharides and xylooligosaccharides (XOSs) have also recently been shown to have promising effects on beneficial commensal microbes and health outcomes (Childs et al. 2014, Goffin et al. 2011, Likotrafiti et al. 2014).

Lactobacillus and *Bifidobacterium* species are the prototypical groups of probiotic microbes (Hill et al. 2014), and the molecular mechanisms of their health-promoting attributes have been extensively researched (Turroni et al. 2014, Ventura et al. 2009). Both genera are gram-positive, strictly fermentative, saccharolytic microorganisms that belong to the phyla Firmicutes and Actinobacteria, respectively. *Lactobacillus* species are low-G+C, facultative anaerobes that metabolize monosaccharides via the Embden-Meyerhof pathway, phosphoketolase pathway, or both. Nutritionally fastidious in nature, *Lactobacillus* species thrive mostly in carbohydrate-rich environments, including milk, cereals, plants, and mucosal surfaces (oral, GIT, and reproductive tracts) of animals and humans, and most probiotic and gut-associated species reside in the host upper GIT. *Bifidobacterium* species, by contrast, are high-G+C, obligate anaerobes and metabolize monosaccharides via the fructose-6-phosphate shunt (bifid shunt). They are found almost exclusively in the mammalian lower GIT and are among the dominant gut colonizers of newborns and breast-fed infants. The accumulating genome sequence data of lactobacilli and bifidobacteria made it evident that most of the probiotic and gut commensal species are endowed with broad genetic capacity predicted to metabolize various complex carbohydrates (Altermann et al. 2005, Lee & O'Sullivan 2010, Schell et al. 2002, Sela et al. 2008). For example, the genomes of *Bifidobacterium longum* NCC2705 and DJO10A harbor 7 and 11 gene clusters, respectively, dedicated to the transport and catabolism of a diverse range of oligosaccharides. This genome feature reflects the specialized adaptation of these microorganisms to their host environments, where complex carbohydrate sources, either host- [e.g., mucin, glycogen, or human milk oligosaccharides (HMOs)] or nonhost-derived (i.e., plant polymers), are abundant.

Genome mining, coupled with functional studies of the glycobiome in probiotic microbes, has begun to elucidate the genetic complements and enzymatic pathways involved in the utilization of these complex carbohydrates. The depolymerization of these oligosaccharides often involves

the interplay of several glycosyl hydrolases (GHs) located intra- and/or extracellularly, as well as specific transporters dedicated to the uptake of intact or hydrolyzed substrate intermediates. In most cases, the genes encoding the transporter components and the associated catabolic enzymes for the same family substrates [e.g., carbohydrates within a range of degrees of polymerization (DP), similar monosaccharide constituents, or linkages] are clustered in conserved modules and coregulated as single operons.

This review summarizes our current knowledge of the genetic mechanisms involved in the catabolism of FOS, GOS, HMO, and several emerging prebiotic candidates among species of *Lactobacillus* and *Bifidobacterium*. Genomic evidence supporting the impact of host niche adaptation on carbohydrate transport and metabolism is also discussed.

2. FRUCTOOLIGOSACCHARIDE METABOLIC PATHWAYS

Naturally present in some edible plants such as Jerusalem artichoke tubers, chicory roots, and onions, FOSs are linear fructose oligomers commercially produced from the hydrolysis of the fructan polymer inulin (e.g., Raftilose) or synthesized from sucrose (e.g., Neosugar, Actilight) via transfructosylation by β-fructofuranosidases (β-FFases; EC 3.2.1.26) or β-D-fructosyltransferases (EC 2.4.1.9) (Hidaka et al. 1988, Niness 1999). Enzymatic synthesis of FOS from sucrose generates oligomers consisting of a glucose monomer (G) linked α-1,2 to two or more β-2,1-linked fructosyl units (F), forming a mixture of 1-kestose (GF_2), nystose (GF_3), and 1^F-fructofuranosyl nystose (GF_4), collectively referred to as GF_n-type FOS or short-chain FOS (Oku et al. 1984) (**Figure 1**). Another type of FOS, known as FF_n-type FOS, is generated by partial hydrolysis of chicory-derived inulin using endoinulinases and characterized by a DP of 2 to 10, with an average DP of 4 (Niness 1999).

2.1. Diversity of Fructooligosaccharide Utilization Pathways Among *Lactobacillus* Species

Researchers first identified and characterized GH32 family β-FFases that hydrolyze FOS in *Bifidobacterium* species in the early 1990s (Imamura et al. 1994, Muramatsu et al. 1992). However, the genetic mechanisms of FOS utilization were not unraveled in *Lactobacillus* species until the emergence of genome sequencing and microarray transcriptomic technologies during the past decade (Barrangou et al. 2003, Goh et al. 2006). Among the *Lactobacillus* species that metabolize oligofructose, FOS utilization appears to occur via one of two catabolic pathways: (*a*) The substrate is transported intact and hydrolyzed by a cytoplasmic GH32 β-FFase, or (*b*) extracellular hydrolysis of substrates is catalyzed by a cell surface–associated GH32 β-FFase, followed by subsequent uptake of the hydrolytic products (i.e., fructose, sucrose, and glucose) via one or more transporters. The majority of FOS-utilizing *Lactobacillus* and *Bifidobacterium* species possess dedicated transporters and intracellular β-FFase for the catabolism of mainly low-DP FOS substrates.

In *Lactobacillus acidophilus*, the transport and hydrolysis of FOS are mediated by an adenosine triphosphate (ATP)-dependent binding cassette (ABC)-type transporter and an intracellular β-FFase, respectively (Barrangou et al. 2003) (**Figure 2**). This pathway is encoded by a multiple sugar metabolism (*msm*) operon with a genetic organization resembling the *msm* operons found in *Streptococcus* species and consisting of a LacI family transcriptional regulator (*msmR*), an ABC transporter (*msmEFGK*), a β-FFase (*bfrA*), and a sucrose phosphorylase. Expression of the operon is under catabolite repression and is specifically induced by FOS and sucrose. Interestingly, based on the NCBI microbial genome database to date (**http://www.ncbi.nlm.nih.gov/genome/browse/**, accessed June 2014), homologous *msm* operons were identified exclusively in *Lactobacillus crispatus*

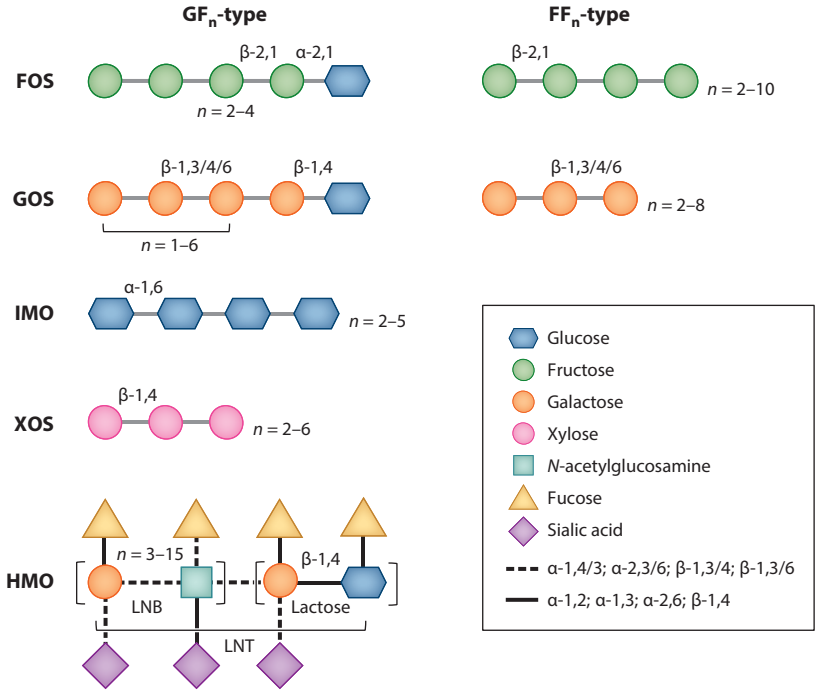

Figure 1

Structural composition of prebiotic oligosaccharide compounds and HMO depicting differences in monomer constituents and glycosidic linkages. Structure of HMO modified from Sela & Mills (2010). Abbreviations: FF$_n$, FOS (generated from partial hydrolysis of inulin) consisting of 2–10 fructosyl units; FOS, fructooligosaccharide; GF$_n$, FOS (synthesized from sucrose) consisting of a mixture of a glucose monomer linked to 2–4 fructosyl units; GOS, β-galactooligosaccharide; HMO, human milk oligosaccharide; IMO, isomaltooligosaccharide; LNB, lacto-*N*-biose I; LNT, lacto-*N*-tetraose; XOS, xylooligosaccharide.

(strain EMLC-1 and ST1), human vaginal isolates of *Lactobacillus jensenii* [strain 269–3 and MD IIE-70(2)], and a vaginal isolate of *Lactobacillus delbrueckii* subsp. *bulgaricus* (strain PB2003/044-T3-4), suggesting that these strains are also likely capable of metabolizing FOS. Because the operon was not detected in other sequenced *L. delbrueckii* strains predominantly associated with dairy fermentation environments, the presence of the *msm* operon in the latter strain is rather intriguing. This indicates either a selective pressure for utilizing FOS-related substrates in the host environment or that the *msm* operon may also be involved in the utilization of other classes of oligosaccharide substrates.

In *Lactobacillus plantarum*, FOS is internalized via a sucrose phosphoenolpyruvate–dependent phosphotransferase system (PTS), and hydrolysis is catalyzed by a cytoplasmic β-FFase (Saulnier et al. 2007). The sucrose PTS EIIBCA and β-FFase genes are divergently oriented and clustered in a single locus, along with an additional three genes coding for a fructokinase, a sucrose operon repressor, and an α-glucosidase. Interestingly, *L. plantarum* selectively grew on GF$_n$-type FOS but not FF$_n$-type FOS. In addition, shorter chains of GF$_n$-type FOS were preferentially utilized. These results reflect the specificity and capacity of the sucrose PTS.

Comparative genomics between a human (ATCC 25644) and a bovine isolate (ATCC 27782) of *Lactobacillus ruminus* revealed a novel FOS utilization operon that is specific to the human isolate (O'Donnell et al. 2011). The operon encodes a β-FFase (BfrA) and a cognate oligosaccharide H^+

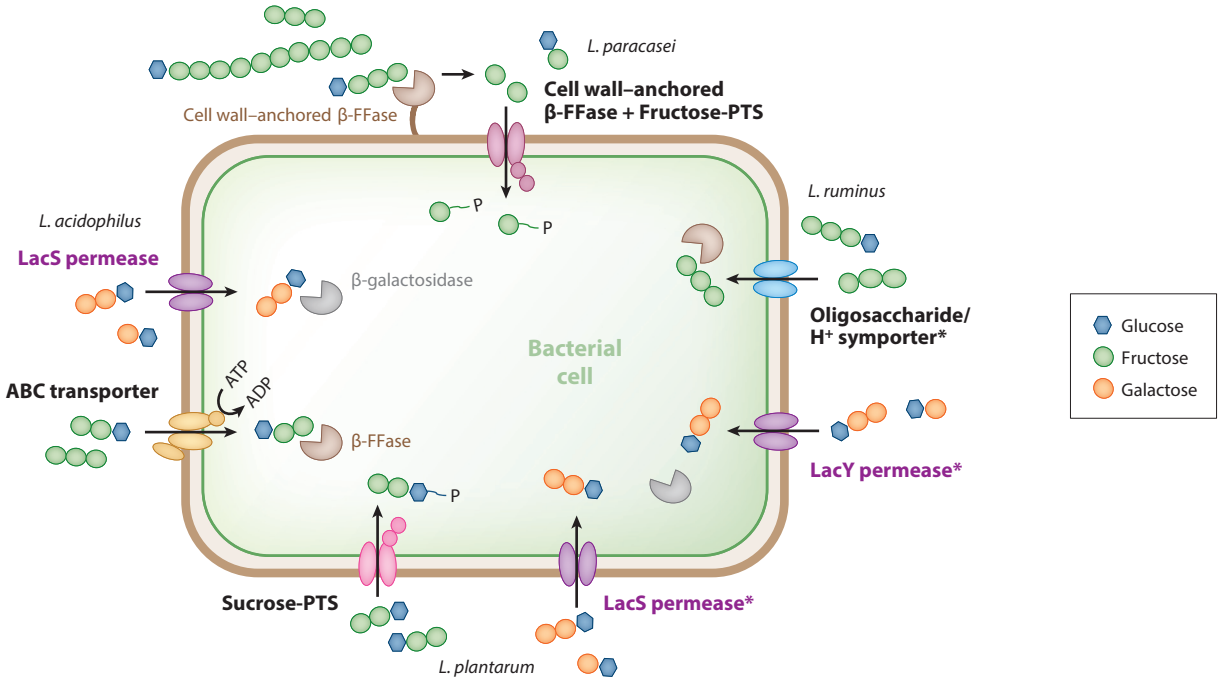

Figure 2

FOS and GOS catabolic pathways identified among *Lactobacillus* species. The diagram highlights the diversity of transport systems (labeled in bold *black* text) and catabolism (intracellular versus extracellular hydrolysis by β-FFase) for FOS. Translocation of GOS by GPH family lactose permeases (LacS or LacY labeled in bold *purple* text) and hydrolysis mediated by intracellular β-galactosidases are also depicted for *L. acidophilus*, *L. plantarum*, and *L. ruminus*. No LacS ortholog was identified among species from the *L. casei-L. paracasei* group. An asterisk (*) denotes transport systems predicted based on in silico analysis. Abbreviations: ABC, ATP-dependent binding cassette; ADP, adenosine diphosphate; ATP, adenosine triphosphate; β-FFase, β-fructofuranosidase; FOS, fructooligosaccharide; GOS, β-galactooligosaccharide; GPH, galactoside-pentose-hexuronide; P, phosphate group; PTS, phosphotransferase system.

symporter [a major facilitator superfamily (MFS) transporter] that were predicted to translocate and hydrolyze FF_n-type FOS as well as a FOS-enriched inulin mixture. The fact that both FOS substrates did not support the growth of ATCC 27782 further substantiates the function of this operon in FOS utilization by the human isolate of *L. ruminus*. Two additional bovine strains in the study exhibited weak to moderate growth on FOS substrates. The authors predicted that the uptake of FOS in these strains was mediated by sucrose PTS transporters.

In contrast to the aforementioned pathways in lactobacilli, *Lactobacillus paracasei* 1195 has adopted a rather different strategy for metabolizing FOS: A cell wall–anchored β-FFase (FosE) mediates extracellular hydrolysis of FOS substrates (Goh et al. 2006) (**Figure 2**). Extracellular hydrolysis of FOS and inulin has been previously reported in another strain of *L. paracasei*, *Lactobacillus pentosus*, and other species such as *Streptococcus mutans* and *Streptomyces exfoliates* (Burne & Penders 1994, Makras et al. 2005, Paludan-Müller et al. 2002, Saito et al. 2000). The genetic determinants of the pathways in most of these microbes remain to be identified. The presence of an LPQAG cell wall–anchoring motif in the FosE β-FFase precursor, coupled with biochemical experiments, established the cell surface localization of FosE in *L. paracasei*, indicating that FOS is hydrolyzed extracellularly in an exo-type fashion, followed by subsequent uptake of the hydrolytic

products (consisting mainly of fructose) via fructose PTS transporters (Goh et al. 2007). Both FosE and a fructose PTS transporter (FosABCDX) are encoded by the *fosRABCDXE* operon. Sequence conservation between the *fos* operon and the putative levanase (*lev*) operons in *Lactobacillus casei* ATCC 334 and BL23 strains, as well as the exclusive presence of FosE orthologs among *L. casei* and *L. paracasei* strains, suggests that FOS utilization via extracellular hydrolysis could be a defining characteristic of the *L. casei-L. paracasei* group. Expression of the *fos* operon was repressed by glucose, with cells exhibiting a diauxic growth pattern on FOS in the presence of glucose. Interestingly, inactivation of the *fosE* gene abolished growth of *L. paracasei* not only on both GF_n- and FF_n-type FOS but also on sucrose, inulin, and a fructan polymer with β-2,6 fructosyl linkages, levan. In parallel, heterologous expression of FosE in the non-FOS-fermenting probiotic strain *Lactobacillus rhamnosus* GG, which possesses functional fructose PTS transporters, enabled the recombinant strain to grow efficiently on GF_n- and FF_n-type FOS, sucrose, inulin, and levan (Goh et al. 2007).

From the evolutionary perspective, one may question how and why different *Lactobacillus* species have evolved to adopt such diverse transport mechanisms (ABC, PTS, or MFS transporters) and catabolic pathways (intracellular versus extracellular hydrolysis) for specific types of oligosaccharide substrates (**Figure 2**). The sequestering of FOS in its intact form for inclusive intracellular catabolism eliminates cross-feeding and confers an advantage in a nutrient-competitive environment. On the other hand, bacteria in the *L. casei-L. paracasei* group, as primary degraders, have an extracellular FOS degradation mechanism that potentially provides cross-feeding of the hydrolytic products generated by the cell surface β-FFase. Nonetheless, this system confers versatility in utilizing longer-chain and more complex prebiotic β-fructoside substrates in mixed linkage types that are not metabolized by other lactobacilli because of the restricted capacity of most transporter systems. This latter strategy may be considered rational for establishing a mutually beneficial or protocooperative relationship with other microbiota members in a niche where complex fructoside substrates are naturally abundant.

2.2. Fructooligosaccharide Utilization in *Bifidobacterium* Species

The ability of bifidobacteria to ferment FOS, specifically shorter-chain oligofructose, is a universal phenotype (Rossi et al. 2005). The general assumption is that bifidobacteria also degrade long-chain fructans such as inulin because of their diverse sugar metabolic gene repertoire and specialized niche in the colon, where complex carbohydrates are abundant. Unexpectedly, most *Bifidobacterium* species grow poorly on inulin, and extracellular enzymes with specificities for long-chain fructans (DP > ~8) are rare among bifidobacteria (Rossi et al. 2005), suggesting their preference for shorter-chain FOS substrates. Overall, the genetic mechanisms and regulation of FOS utilization in this genera are less well defined, particularly for the transport systems involved in the uptake of these oligomers. In the majority of species, FOS fermentation relies on intracellular β-FFase and dedicated permeases or ABC transporters.

The *cscA* gene encoding β-FFase has been determined in *Bifidobacterium adolescentis* (Omori et al. 2010), *Bifidobacterium breve* (Ryan et al. 2005), *Bifidobacterium lactis* (Ehrmann et al. 2003, Janer et al. 2004), and *B. longum* (Kullin et al. 2006). In *B. breve*, the β-FFase is encoded in a FOS-induced operon, along with a putative sucrose permease gene, *cscB*. Researchers have also identified similar operons in *B. longum* and *B. lactis* (Kullin et al. 2006, Ryan et al. 2005). The operon is apparently induced by FOS of the GF_n type but not the FF_n type. Interestingly, CscA hydrolyzed the β-2,1 linkage between the glucose and fructose moieties of oligofructose but not the β-2,1 linkage between two fructose moieties within the same substrate, leaving behind chains of fructose molecules as residual hydrolytic products.

Of the 19 carbohydrate metabolic operons predicted within the genome of *B. longum* NCC2705, microarray transcriptome analysis revealed the induction of nine sugar transport systems during growth on FF_n-type FOS (Parche et al. 2007). These include seven ABC transporters and two MFS-type permeases. Whereas the aforementioned *cscA* gene was not expressed in *B. longum* NCIMB 702259^T during growth on FF_n-type FOS (Kullin et al. 2006), the entire *cscABR* operon was upregulated by the same FOS substrates in the NCC2705 strain. Most of the FOS-induced transport systems were also inducible by other substrates such as lactose and maltose, indicating multiple substrate specificities of the transporters that likely enabled a broader range of sugar utilization and differential regulation based on nutrient competitiveness.

Studies of FOS and inulin fermentation with mixed fecal cultures demonstrated that other fecal bacterial species served as primary degraders of inulin; they provided cross-feeding of monosaccharides and short-chain oligofructose for bifidobacteria (Rossi et al. 2005). More importantly, bifidobacterial growth on FOS or inulin did not differ significantly in the mixed fecal populations. This indicates that bifidobacteria could still grow competitively from scavenging partially hydrolyzed substrates from the primary degraders and likely reflects a mutual relationship between these bacteria for nutrient metabolism in vivo.

3. β-GALACTOOLIGOSACCHARIDE METABOLIC PATHWAYS

Structurally based on lactose and with minor similarities to the core molecules of HMO, GOS was developed as a mimetic for HMO with the aim to simulate its prebiotic effects and other biological functions (Boehm & Stahl 2007, Sela & Mills 2010). Sometimes also termed transgalactooligosaccharides, commercial GOSs are synthesized by the transgalactosylation activity of β-galactosidases using lactose, generally in high concentrations, as a starting substrate (Gosling et al. 2010). GOSs are typically composed of terminal lactose at the reducing end linked to one to six galactose moieties $\{[Gal(\beta 1–3/4/6)]_{1-6}Gal(\beta 1–4)Glc\}$, with some GOS mixtures also containing Gal_n-Gal $\{[Gal(\beta 1–3/4/6)]_{\sim 1-7}Gal\}$ (**Figure 1**) as well as branched structures composed of multiple galactose moieties linked to a single glucose moiety at the reducing end. The heterogeneity of the final GOS structures depends highly on the source of β-galactosidase and reaction parameters. Notably, researchers observed that the β-galactosidases from several *Bifidobacterium* species each produced distinct GOS structures and grew more efficiently on the oligosaccharides that were produced by their own β-galactosidases compared to commercial GOS (Rabiu et al. 2001).

3.1. β-Galactooligosaccharide Utilization in *Lactobacillus* Species: The Role of LacS Permease

The molecular mechanism of GOS utilization by probiotic microbes was first described in *L. acidophilus* NCFM, when researchers identified the lactose permease (LacS) as the key player in GOS metabolism (Andersen et al. 2011) (**Figure 2**). Microarray transcriptomic studies revealed GOS specifically induces the *gal-lac* operon that encodes LacS, a galactoside-pentose-hexuronide (GPH) family permease, two cytoplasmic β-galactosidases (GH42 LacA and GH2 LacLM), and enzymes of the Leloir pathway for galactose metabolism. Accordingly, GOS is transported via the GPH-type LacS permease and hydrolyzed by LacA and LacLM into glucose and galactose, which are subsequently metabolized via the glycolytic and Leloir pathways, respectively. The *lac* operon was also inducible in lactose-grown cells (Barrangou et al. 2006), indicating that the *lac* operon in *L. acidophilus* was responsible for the metabolism of lactose, GOS, and potentially other galactosides, such as fractions of HMO. Inactivation of *lacS* abolished growth on GOS, lactose, or

lactitol as sole carbon source. These results established LacS as the sole transporter for GOS and suggested that LacS has divergent and broad substrate specificity for β-galactosides. Investigators previously proposed that GH2 and GH42 β-galactosidases are involved in the degradation of HMO (Marcobal et al. 2010). This further implies the potential involvement of the *gal-lac* operon in HMO utilization, although *L. acidophilus* NCFM was previously shown to exhibit weak but noticeable planktonic growth on HMOs. Interestingly, this gene cluster was also upregulated by bile exposure (Pfeiler et al. 2007), revealing an adaptive combination of gut-evolved traits for bile tolerance and utilization of carbohydrates common in mammals. Evidence that GOS utilization genes are expressed upon bile exposure in the gut has revealed a novel strategy for delivering *L. acidophilus* and GOS as synbiotics in dairy products. GOS acts as an inducer and an immediate nutrient source that signals rapid uptake and metabolism of the prebiotics when cells reach the intestine and thus enhances survivability.

Examination of gene clusters containing *lacS* among sequenced lactobacilli and related lactic acid bacteria (LAB) revealed structural conservation of the *gal-lac* clusters among the acidophilus complex lactobacilli such as *L. crispatus*, *Lactobacillus johnsonii*, and *Lactobacillus helveticus* (Andersen et al. 2011). This suggests that the *gal-lac* gene cluster may also confer upon these species the capability to transport and utilize GOS as a carbon source. The *lac* gene clusters differ markedly in other LAB such as *L. plantarum*, *Lactobacillus reuteri*, *Lactobacillus fermentum*, and *L. bulgaricus*. Interestingly, regardless of the difference in gene organization, *lacS* genes are present, along with *lacA* GH42 β-galactosidases, for all lactobacilli except *L. bulgaricus*, indicating coevolution of LacS and GH42 β-galactosidases. The copresence of *lacS* and *lacA* inclusively within the intestinal-associated acidophilus complex species (all strains of each species are $lacS^{+}$) further indicates lactose and galactosides as important energy sources in the GIT for these lactobacilli, which potentially provide selective advantages over microbiota members that are unable to metabolize complex galactosides. Additionally, the adoption of an MFS-type transporter allows energy-efficient, rapid adaptive transport of GOS and enables scavenging of the substrates in a nutrient-competitive niche. *L. bulgaricus* possesses a *lacZ* GH2 β-galactosidase, and its *lac* operon shares high similarity to that of *Streptococcus thermophilus*, likely due to genetic exchange occurring in their common dairy niche. Differences in gene arrangement and the types of encoded β-galactosidases reflect specific adaptation among these LAB toward the metabolism of a variety of β-galactoside substrates. Paradoxically, *Lactobacillus gasseri*, a probiotic species that is frequently encountered in human milk and the infant gut, does not ferment GOS (J.J. Lee, R.B. Sanozky-Dawes & T.R. Klaenhammer, unpublished data) or HMO in vitro (Ward et al. 2006). *L. gasseri* lacks a *lacS* or *lacA* gene and instead possesses lactose PTSs and phospho-β-galactosidase for lactose metabolism (Azcarate-Peril et al. 2008, Francl et al. 2012). This supports an idea of niche partitioning in which the approximate niches of *Lactobacillus* species within compartments of the small intestine may be dictated by their varying capability to utilize simple versus complex carbohydrates and the specific pathways they use to metabolize the substrates.

Researchers predicted that, similar to *L. acidophilus*, the transport and hydrolysis of GOS and other β-galactosides such as lactose and lactulose by *L. ruminus* ATCC 26544 are mediated by GPH family lactose permeases (LacY) and β-galactosidases (LacZ) (O'Donnell et al. 2011) (**Figure 2**). Two operons of *lacIZY* are present in the human strain ATCC 26544, whereas genes associated with β-galactoside metabolism are completely absent in the bovine strain ATCC 27782. Two additional bovine strains included in the study by O'Donnell et al. (2011) showed lactose- and GOS-positive phenotypes; the genomes of these strains have not been sequenced. This implies that the loss of lactose metabolism capability is not exclusive to bovine strains of *L. ruminus* and is likely the result of specialized adaptation of ATCC 27782 to an environment where milk sugars are scarce.

3.2. Differential β-Galactooligosaccharide Utilization in Bifidobacteria: The Role of Endogalactanase for Extracellular β-Galactooligosaccharide Hydrolysis

Glycoprofiling of GOS consumption revealed differential utilization of GOS substrates among several bifidobacterial species (Barboza et al. 2009). The infant isolates of *B. breve* and *B. longum* subsp. *infantis* both utilized pure GOS (pGOS) substrates more efficiently than the adult isolates of *B. longum* subsp. *adolescentis* and *B. longum* subsp. *longum*. The prior two strains utilized pGOS with DP of 3 to 8, compared to *B. longum* subsp. *adolescentis* and *B. longum* subsp. *longum*, which showed preferential fermentation of pGOS with DP of 3 and 5 to 6, respectively. Garrido et al. (2013) further demonstrated interstrain variation of GOS consumption profiles among 22 strains of *B. longum* subsp. *infantis*. The differential GOS consumption phenotypes among the bifidobacterial strains suggest that differential GOS catabolic systems evolved through host adaptation and, more importantly, provided a basis for targeted GOS enrichment of specific bifidobacterial phylotypes or strains.

B. longum NCC2705 and *B. breve* UCC2003 possess analogous extracellular GH53 cell membrane–bound endogalactanases (GalA) capable of degrading plant-derived galactan. The enzyme liberates galactotriose from galactan polymers with β(1–4) and β(1–3) linkages as well as GOS in an exo-type fashion toward the reducing end of the polymers (Hinz et al. 2005). The resulting galactotriose is imported by the cells via an ABC transporter (GalCDE) and further hydrolyzed by an intracellular GH42 β-galactosidase (GalG) encoded in the same locus (*galCDEGRA*) (O'Connell Motherway et al. 2010). Among strains of *B. breve*, the presence of GalA was also an important determinant for efficient GOS utilization (O'Connell Motherway et al. 2013). The endogalactanase specifically targets GOS with DP > 3 that otherwise were not utilized by strains that do not possess GalA. The partially degraded GOS were primarily imported via the aforementioned GalCDE ABC transporter and hydrolyzed by GalG. In addition to the *galCDEGRA* cluster, growth on pGOS also induced two additional gene loci that each encode a LacS permease and the GosDEC ABC transporter, both associated with a β-galactosidase, LacZ (GH2) and GosG (GH42), respectively. Only inactivation of the *galA*, *galC* (ABC transporter substrate-binding protein), and *galG* genes resulted in impaired growth on pGOS, indicating that the LacS-LacZ and GosDEC-GosG systems play complementary roles in GOS utilization. This is in contrast to the role of LacS in *L. acidophilus* as the sole transporter for GOS, lactose, and lactulose (Andersen et al. 2011), whereas in *B. breve*, LacS is the sole uptake system for only the latter two substrates (O'Connell Motherway et al. 2013). In *B. lactis* Bl-04, GOS specifically induced the expression of two operons encoding (*a*) a putative MFS lactose permease and a GH2 β-galactosidase and (*b*) an ABC transporter and a GH42 β-galactosidase, respectively (Andersen et al. 2013). The genetic architecture of these two operons was similar to that of the *lacSZ* and *gosRDEGC* operons in *B. breve*, respectively, although the proteins shared only moderate sequence homology.

Interestingly, based on the current NCBI protein database, GalA orthologs are found exclusively in strains of *B. breve* and *B. longum*, including *B. longum* DJO10A (BLD_1003). In line with the findings described previously (O'Connell Motherway et al. 2010), analogous *galCDEGRA* operons are present in both *B. longum* NCC2705 and DJO10A, indicating a similar mechanism is involved in GOS utilization by these strains. In *B. longum* NCC2705, the same operon is also associated with the catabolism of lactose and FOS (Parche et al. 2007).

4. METABOLISM OF OTHER POTENTIAL PREBIOTICS

Researchers have extensively investigated novel, nondigestible oligosaccharides, some of which are developed based on the structures of resistant starch- and nonstarch-based plant polysaccharides,

for their potential application as second-generation prebiotic ingredients. Some of these prebiotic candidates, such as glucooligosaccharide and XOS family compounds, selectively enrich beneficial and probiotic gut microbes (Mäkeläinen et al. 2009, 2010; Yen et al. 2011). This section summarizes recent findings on the molecular mechanisms involved in the degradation of these novel prebiotics.

4.1. Glucooligosaccharides

Andersen et al. (2012) recently demonstrated that, in addition to FOS and GOS, *L. acidophilus* encodes an expansive repertoire of enzyme machinery for the catabolism of a broad range of potential prebiotic substrates varying in glycoside linkages and monosaccharide constituents. Differential transcriptomic studies on *L. acidophilus* NCFM have identified putative catabolic pathways for the utilization of eleven prebiotic candidates, including the α-glucooligosaccharides panose (α-D-Glu-α-1,6-α-D-Glu-1,4-D-Glu) and polydextrose as well as β-glucan oligomers derived from the hydrolysis of mixed-linkage β-1,3/β-1,4 β-glucan. All three substrates induced multiple transport systems, most of which are PTS transporters. This study revealed the broad specificities of some of the sugar uptake systems in *L. acidophilus*, irrespective of the monomer constituents or linkage types. For example, the PTS for isomaltose and isomaltulose (LBA0606-0609) was also induced by all three glucan substrates. Another example is the upregulation of a previously established FOS ABC transporter (Barrangou et al. 2003) by polydextrose consisting of mixed-linkage α-glucans, suggesting a broad specificity of the FOS ABC transporter in *L. acidophilus*. The coinduced cytoplasmic glycosidases include a GH4 maltose-6-phosphate glucosidase (LBA1689, MalH), a GH65 maltose phosphorylase (LBA1870, MalP), and a GH1 6-phospho-β-glucosidase II (LBA0726, BglBII), which are predicted to hydrolyze internalized panose, polydextrose, and β-glucan oligomers, respectively.

The GH13 family, which includes many enzymes active on α-glucan substrates, represents the largest GH family encoded in large subsets of *Lactobacillus* and *Bifidobacterium* species (Møller et al. 2014). This reflects the significance of α-glucan metabolism among certain species or strains, particularly those that have adapted to glucan-rich environments. For instance, *L. acidophilus* possesses seven to nine GH13 enzymes, in contrast to the milk-adapted *L. fermentum* strains and the meat-adapted *Lactobacillus sakei*, which have only one and two copies of GH13 hydrolases, respectively (Møller et al. 2014). Recently, Møller et al. (2012) structurally and biochemically characterized a cytoplasmic GH13 subfamily 31 glucan-1,6-α-glucosidase (*La*GH13_31; G16G) in *L. acidophilus* NCFM that confers the hydrolysis of isomaltooligosaccharides (IMOs) consisting of α-1,6-glucooligosaccharides and panose. Purified G16G showed selective specificities toward IMO with DP > 2 and toward panose. Unlike in other related species such as *L. johnsonii* and *L. gasseri*, the G16G-encoded gene (*lba0264*) in *L. acidophilus* exists as a monocistronic unit and is not associated with the putative maltooligosaccharide utilization operon (*lba1864* to *lba1874*), a feature that is also shared by closely related acidophilus complex members, e.g., *Lactobacillus amylovorus* and *L. crispatus*. Expression of *lba0264* and the putative maltose operon in *L. acidophilus* was upregulated in response to IMO and maltotetraose (an α-1,4 glucooligosaccharide), suggesting a coregulation of catabolic pathways for α-1,4 and α-1,6 glucan substrates. Given that the *lba0264* homologs are colocalized with the maltooligosaccharide operon in other *Lactobacillus* species (see above), and the metabolism of both α-1,4 and α-1,6 oligosaccharides appears to be coregulated and involves common enzymes (e.g., MalP for maltose phosphorolysis), the ABC transporter encoded in the operon likely also plays a role in the import of IMO (Abou Hachem et al. 2013, Møller et al. 2012). By contrast, the genetic organization of IMO metabolic genes in *Bifidobacterium* is strikingly different, in that the

α-1,6-glucosidase gene is clustered within the operon for the catabolism of α-1,6-galactosides (e.g., raffinose and stachyose) (Abou Hachem et al. 2013). In addition, carbohydrate transcriptome analysis of *B. lactis* Bl-04 revealed the coinduction of this operon by both IMO and α-1,6-galactosides, demonstrating the potential dual specificities of the ABC transporter encoded in the operon (Andersen et al. 2013).

The ability to degrade starch appears to be relatively species specific among bifidobacteria (Ryan et al. 2006). Notably, species that demonstrate extracellular starch hydrolysis activities include *B. breve*, *Bifidobacterium pseudolongum*, and *Bifidobacterium thermophilum*. In *B. breve* UCC2003, starch hydrolysis is mediated by a unique cell wall–anchored class II bifunctional amylopullulanase (ApuB) that exhibits specificities for α-1,4 and α-1,6 glucosidic linkages at its N-terminal α-amylase and C-terminal pullulanase domains, respectively (O'Connell Motherway et al. 2008). Mutational analysis further supports the role of ApuB in the utilization of starch and starch-like polysaccharides, e.g., glycogen and pullulan.

4.2. Xylooligosaccharides

XOSs are composed of β-(1,4)-linked xylosyl oligomers commonly derived from enzymatic hydrolysis of xylan polysaccharides extracted from plant cell walls. GHs that target XOS- and arabino-XOS (XOS decorated with arabinosyl side chains and produced from primary degradation of arabinoxylan by gut xylanolytic genera, e.g., *Bacteroides* and *Roseburia*) have been identified in several *Bifidobacterium* species, including *B. breve*, *B. lactis*, *B. adolescentis*, and *B. bifidum* (Amaretti et al. 2013, Gilad et al. 2010, Lagaert et al. 2010, Shin et al. 2003, Zeng et al. 2007). In addition, ABC transporters for the uptake of XOS have been found exclusively in bifidobacteria among members of the gut microbiota (Ejby et al. 2013). In *B. lactis* BB-12, microarray transcriptomic and proteomic analyses during growth on XOS revealed the upregulation of an ABC transporter with several intracellular XOS-degrading and metabolic enzymes (Gilad et al. 2010). Based on the proposed XOS catabolic pathway, XOS (DP of 2 to 6) transported via the ABC system were hydrolyzed by endo-1,4-β-xylanases and β-xylosidases. The former cleaves XOS randomly, whereas the latter releases D-xylose from XOS.

Ejby and coworkers (2013) further demonstrated that *B. lactis* Bl-04 was capable of importing both XOS and arabino-XOS via an ABC transporter substrate-binding protein (*Bl*AXBP) that recognizes both arabinosyl-decorated and undecorated XOS with preferential DP of 3 to 4. Detailed crystallography work on *Bl*AXBP revealed oligosaccharide substrate recognition in two opposite orientations and a spacious binding pocket that accommodates arabinosyl decorations at different main chain positions, providing structural plasticity and broader specificity. The authors hypothesized that the substrate preference specificity of *Bl*AXBP enables cross-feeding of XOS released by primary degraders and provides a strategy to eliminate nutrient competition from other microbiota, i.e., *Bacteroides* species, which prefer larger xylan polymers, and Firmicutes capable of utilizing only xylobiose (DP of 2) and undecorated XOS substrates.

The XOS utilization pathway of *B. lactis* Bl-04 is encoded by a twelve-gene operon consisting of the ABC transporter components, a GH43 β-xylosidase, two GH43 arabinofuranosidases, two esterases, and enzymes required to convert metabolic intermediates for entry into the bifid-shunt pathway (Andersen et al. 2013). The presence of arabinofuranosidases and carbohydrate esterases indicates the capability of Bl-04 to remove arabinosyl and acetyl or feruloyl side chains from intracellular arabino-XOS substrates. Multiplication of the arabinofuranosidase, esterase, and xylosidase genes among bifidobacterial species is common and contributes to the diversity of the operon architecture. This diversity reflects specialized adaptation among the species or strains to metabolize specific types of decorated XOS with varying degrees of complexity (Andersen et al. 2013).

5. GENETIC MECHANISMS OF HUMAN MILK OLIGOSACCHARIDE UTILIZATION BY INFANT BIFIDOBACTERIA

HMO, ranked the third most abundant solid component in breast milk after lactose and lipids, represents a complex population of nondigestible oligosaccharides composed of five monosaccharides: D-glucose (Glc), D-galactose (Gal), *N*-acetylglucosamine (GlcNAc), L-fucose (Fuc), and sialic acid (*N*-acetylneuraminic acid; Neu5Gc) (Bode 2006) (**Figure 1**). Lactose (Galβ1–4Glc) at the reducing end is linked β1–3 to ≤15 repeat units of either lacto-*N*-biose I (LNB; Galβ1–3GlcNAc) or *N*-acetyllactosamine (Galβ1–4GlcNAc), forming type 1 and 2 HMOs, respectively. In addition to the length variation of the LNB repeats, structural diversity of the milk oligosaccharides is also attributed by terminal fucosylation (via α1–2/3/4 linkages) and sialylation (via α2–3/6 linkages) of the HMO core structures at the nonreducing ends, which are thought to restrict access to microbial degradation of the core structures (Bode 2006, Sela & Mills 2010). The ability to grow on HMO or LNB is a common trait among infant gut–associated species of *B. longum* subsp. *infantis* and *B. bifidum*, albeit to a lesser extent among *B. longum* subsp. *longum* and *B. breve* strains. By contrast, this phenotype is generally absent in the adult-associated species, i.e., *B. adolescentis* and *Bifidobacterium catenulatum* (Asakuma et al. 2011, Ward et al. 2007, Xiao et al. 2010).

The predominance of type 1 oligosaccharides is a feature in milk and colostrum from humans that is distinct from other mammals, including anthropoids, which are biased toward type 2 oligosaccharides (Urashima et al. 2012). Not surprisingly, the catabolic pathway for the utilization of the disaccharide LNB, the main building block of type 1 HMOs, is conserved in all infant-derived species of *B. bifidum*, *B. longum* subsp. *infantis*, *B. longum* subsp. *longum*, and *B. breve* (Xiao et al. 2010). The so-called GNB/LNB pathway, first identified in *B. bifidum* and *B. longum*, is encoded in an operon consisting of genes involved in the uptake and intracellular hydrolysis of LNB as well as GNB (galacto-*N*-biose; Galβ1–3GalNAc), the building block for mucin sugars (Kitaoka et al. 2005, Nishimoto & Kitaoka 2007). Import of LNB or GNB is mediated by an ABC transporter (GltABC). Subsequently, substrate catabolism is mediated by a series of enzymes, including a lacto-*N*-biose phosphorylase (LNBP) that cleaves LNB and GNB disaccharides and two enzymes analogous to the Leloir pathway enzymes for galactose metabolism. Gonzalez et al. (2008) found the GNB/LNB pathway operon in *B. longum* LMG 13197 to be upregulated during growth in human milk, along with genes involved in lactose and GlcNAc metabolism and putative genes encoding cell surface type 2 glycoprotein-binding fimbrae potentially involved in gut epithelial adherence. Specific induction of the carbohydrate metabolic genes further substantiates the role of the unique and complex HMOs on the bifidogenic effect of human milk.

Owing to the inherent structural complexity of HMOs, additional enzymatic pathways are present in bifidobacteria that are predicted to facilitate their access to the core structure of the oligosaccharides. *B. longum* subsp. *infantis*, regarded as the archetypical HMO phylotype, harbors a unique 43-kb gene cluster that encodes an array of glycosidases, ABC transporters, and extracellular oligosaccharide solute-binding proteins tailored for HMO utilization (Sela et al. 2008). This cluster, along with two of four other HMO-related loci, is conserved among all HMO^+ *B. longum* subsp. *infantis* strains but is absent in the closely related *B. longum* subsp. *longum*; these bacteria are considered specialized degraders of plant-derived glycans from their adult host diet (LoCascio et al. 2010, Sela et al. 2008). Interestingly, two of the HMO-linked clusters encoding fucosidases were predicted to arise from recent gene duplication. One of these clusters also appeared to replace the plant polysaccharide utilization cluster at the corresponding locus in closely related *B. longum* subsp. *longum*, reflecting a selective pressure for *B. longum* subsp. *infantis* toward the metabolism of mammalian glycans (Sela et al. 2008). All catabolic enzymes encoded in the HMO utilization clusters, including fucosidases, sialidase, β-galactosidase, and

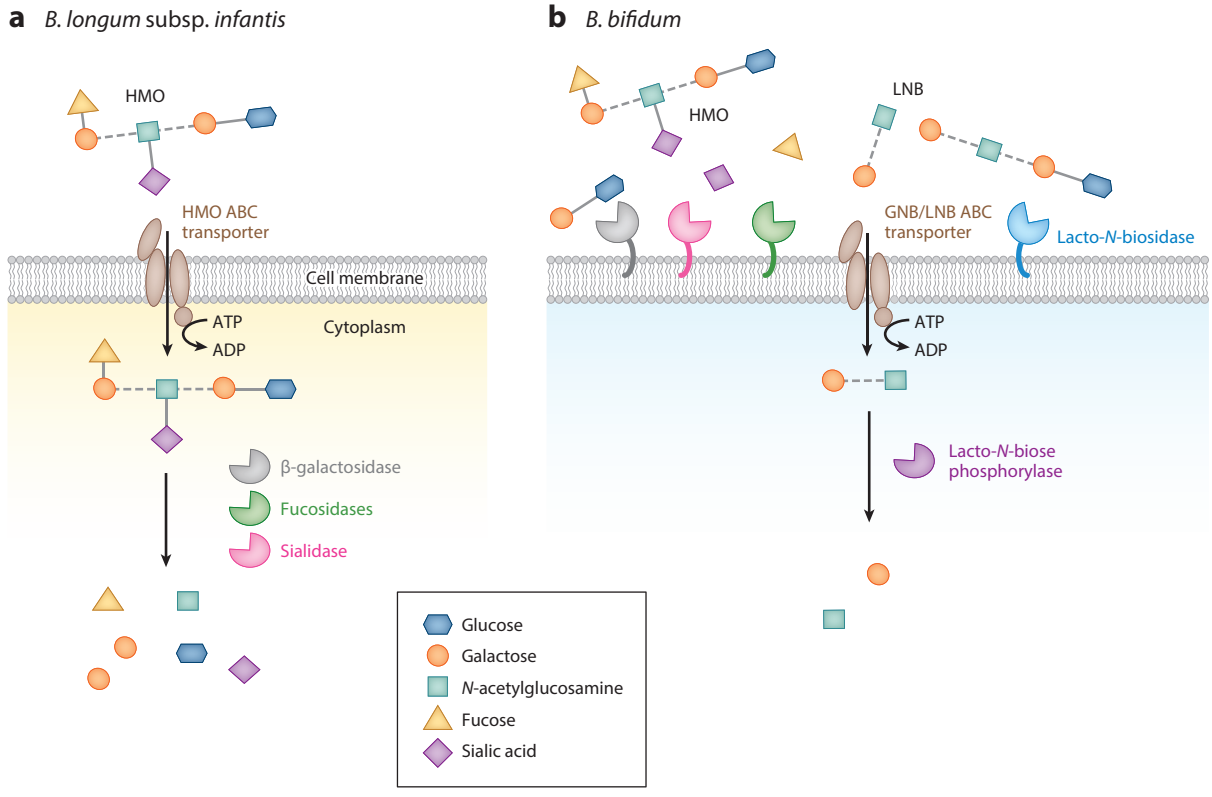

Figure 3

Proposed pathways for HMO utilization in *Bifidobacterium longum* subsp. *infantis* and *Bifidobacterium bifidum*. (*a*) In *B. longum* subsp. *infantis*, HMO substrates are transported intact via an ABC transporter, and subsequent hydrolysis is mediated by various cytoplasmic glycosidases. (*b*) In contrast, *B. bifidum* possesses cell membrane–bound glycosidases that depolymerize HMOs extracellularly, including a lacto-*N*-biosidase that cleaves HMOs and generates LNB hydrolytic products. LNB is imported via the GNB/LNB ABC transporter and further hydrolyzed by lacto-*N*-biose phosphorylase in the GNB/LNB pathway. Abbreviations: ABC, ATP-dependent binding cassette; ADP, adenosine diphosphate; ATP, adenosine triphosphate; GNB, galacto-*N*-biose; HMO, human milk oligosaccharide; LNB, lacto-*N*-biose I.

β-*N*-acetylhexosaminidase, are intracellular based on their lack of secretion signals. The cytoplasmic localization of these glycosidases and the observed preferential utilization of short HMO substrates (DP of ≤7), especially lacto-*N*-tetraose (Galβ1–3GlcNAcβ1–3Galβ1–4Glc), provide corroboratory evidence that HMOs are imported via the ABC transporters and hydrolysis occurs intracellularly (Sela & Mills 2010) (**Figure 3**). *B. longum* subsp. *infantis* does not possess a homolog of lacto-*N*-biosidase for the liberation of LNB from HMO, although an intact GNB/LNB pathway is present for the catabolism of LNB (Sela et al. 2008). Instead, it appears to rely on two novel β-galactosidases of the GH42 (Bga42A) and GH2 (Bga2A) families that differentially target type 1 and type 2 HMOs, respectively (Yoshida et al. 2012). Accordingly, internalized HMOs are degraded by Bga42A from the nonreducing end in an exo-type fashion.

On a different note, HMOs are hydrolyzed extracellularly by *B. bifidum* with the combined action of an arsenal of membrane-bound enzymes. These include α-fucosidases and 2,3/6-α-sialidases that initiate removal of the decorated fucose and sialic acid residues and subsequent

liberation of LNB from HMOs by a membrane-anchored lacto-*N*-biosidase (Ashida et al. 2009, Katayama et al. 2004, Wada et al. 2008) (**Figure 3**). The LNB intermediates are imported by an ABC transporter and further metabolized via the GNB/LNB pathway. One would expect that this extracellular degradation strategy circumvents limitation of transporter capacity and provides a competitive advantage for *B. bifidum* by allowing it to utilize a wider range of HMO substrates. Interestingly, the microorganism did not incorporate all degraded metabolites, such as fucose and galactose, during planktonic growth on HMOs (Asakuma et al. 2011). It remains to be determined whether similar HMO consumption patterns occur in vivo and, if so, whether *B. bifidum* evolves a cross-feeding strategy to establish beneficial mutualism with other gut commensals.

Milk oligosaccharides share many common *O*-glycan structural motifs of the intestinal epithelial mucin and glycoconjugates. Beyond functioning as a protective barrier of the GIT, the intestinal epithelial mucus layer, specifically the outer layer, displays sites for adhesion and colonization of the gut commensals. In addition, mucin glycoproteins represent important, nutrient-rich substrates secreted by the host as a strategy to minimize nutrient fluctuation for the gut microbiota and maintain species composition (Backhed et al. 2005, Johansson et al. 2011). Among bifidobacteria, only *B. bifidum* strains showed efficient growth when mucin was provided as the sole carbon source (Turroni et al. 2010). Accordingly, researchers have proposed that part of the extracellular enzyme repertoire (e.g., β-galactosidase and *N*-acetyl-β-hexosaminidases) for hydrolysis of HMOs and the GNB/LNB pathway present in *B. bifidum* plays a role in mucin utilization (Kitaoka et al. 2005, Turroni et al. 2010). Proteomic analysis confirmed that the GNB/LNB pathway was induced when *B. bifidum* was cultivated on mucin (Turroni et al. 2010). Furthermore, impaired growth on mucin in some *B. bifidum* strains has been correlated with the lack of a functional GNB/LNB ABC transporter. The convergence of HMO and mucin utilization pathways in *B. bifidum* manifests an ecological fitness strategy for nutrient acquisition from milk and host oligosaccharides and, consequently, colonization in the infant GIT.

6. CONCLUSION AND FUTURE PERSPECTIVES

Probiotic species of *Bifidobacterium* and *Lactobacillus* possess a remarkably diverse glycobiome for the metabolism of varied oligosaccharides and complex carbohydrates. In general, the carbohydrate gene repertoires in certain species or strains often dictate their differential substrate preference based on chain length, monomer constituents, glycosidic linkages, and the overall structural complexity of the oligosaccharides (e.g., presence of decorated side chains). These gene repertoires in turn are shaped by gut nutrient adaptation and, consequently, niche differentiation of the microbial population that cohabits the GIT. In a simplified view, the majority of gut *Lactobacillus* species encode multiple broad-specificity transporters and cytoplasmic enzymes, enabling them to thrive in small-intestinal niches rich in simple sugars and nondigestible oligosaccharides. By contrast, as specialized commensals in the large intestine, where complex carbohydrates are the major carbon source, bifidobacteria are genetically equipped with extracellular GHs and carbohydrate scavenging machinery that confer utilization and cross-feeding of higher-order oligosaccharides. The evolution toward greater specialization of substrate preference and resource partitioning has been proposed as a niche adaptation mechanism of these microbes to minimize nutrient competition while also allowing metabolic syntrophy (cross-feeding) with other gut commensals (Ejby et al. 2013, Tannock et al. 2012).

Inter- and intraspecies comparisons of lactobacilli and bifidobacteria have provided further evidence for prebiotic utilization as an important niche adaptation to the GIT. For example, genome comparisons between probiotic *L. acidophilus* and the closely related *L. helveticus*, a

dairy-domesticated species, revealed the absence of gene loci involved in the catabolism of FOS, polydextrose, panose, and raffinose, along with genes associated with bile salt tolerance and epithelial adhesion in the latter species (Andersen et al. 2011, 2012). In *B. longum*, the HMO utilization phenotype defines the phylotype delineation between the subspecies *longum* and *infantis* (Sela & Mills 2010). The infant-type *B. longum* subsp. *infantis* encodes multiple and duplicated gene clusters specialized for efficient catabolism of HMO. One of these HMO-linked clusters appeared to evolve from a plant polysaccharide utilization gene cluster in the corresponding adult-type *B. longum* subsp. *longum*, emphasizing the genomic adaptation of these subspecies toward their host diets. In vivo transcriptome studies on gut microbiota in response to prebiotic substrates consistently showed carbohydrate metabolic genes as the major induced functional gene category, indicative of active utilization of complex carbohydrates among the microbial population. In a study by Klaassens et al. (2011), when adults were fed a prebiotic mixture containing GOS, long-chain FOS, and pectin hydrolysate, genes related to plant polysaccharide degradation were significantly induced among bifidobacteria. In a more recent functional metagenomic study, metagenome libraries of the fecal and ileum mucosa microbiota were screened for degradation activities on several prebiotic substrates, including FOS, GOS, and XOS (Cecchini et al. 2013). In particular, the ileal microbiota, including nonculturable and unknown species, showed efficient hydrolysis of the prebiotics. Sequencing of the metagenomic DNA inserts revealed novel pathways and diverse GHs involved in prebiotic catabolism. Furthermore, the study indicated a nonspecific target effect of the prebiotic compounds in the gut ecosystem.

Researchers in the prebiotic field should address frequently the impact of prebiotic applications on adaptive carbohydrate utilization by pathogenic microbes. A growing body of evidence has shown the dissemination of FOS utilization gene clusters among pathogenic strains of *Escherichia coli* (Dolejska et al. 2014; Le Bouguénec & Schouler 2011; Porcheron et al. 2011, 2012; Schouler et al. 2009) and the ability of various pathogenic streptococcal species to utilize FOS (Hartemink et al. 1995, Linke et al. 2013). Moreover, considering the ability to metabolize lactose is widespread among microbes, it remains to be determined whether pathogenic species or other nontargeting commensals that possess LacS are also capable of utilizing GOS compounds and, more importantly, whether probiotic species are capable of outcompeting these other microbes in the presence of prebiotic substrates (J.J. Lee, R.B. Sanozky-Dawes & T.R. Klaenhammer, personal communication).

Overall, the advancement of genome sequencing technology and genetic tools has not only enabled us to predict, functionally analyze, and compare the genetic elements involved in carbohydrate catabolism by lactobacilli and bifidobacteria, but also allowed us to fully exploit the saccharolytic potential of these microbes for tailoring of effective prebiotics. We are still far from fully understanding the genetic regulation of prebiotic metabolic pathways and the precise molecular mechanisms of how prebiotics modulate the microbiota composition in vivo. Ongoing studies using in vitro intestinal fermentation models, in vivo animal models, and metagenomic analysis are aimed at comprehending how prebiotic and plant polysaccharide compounds impact and shape the host microbiota as well as the metabolic syntrophy that occurs between probiotic microbes and other gut commensals. These experimental systems will be ideal for investigating the potential horizontal transfer and dissemination of prebiotic catabolic gene clusters among gut commensals. Our evolving understanding of the mechanistic interactions of probiotics and prebiotics, especially in a mixed-culture system, will provide the molecular basis for targeting prebiotics to specific, beneficial, commensal populations; the development of novel prebiotic compounds with high specificity; and the design of effective probiotic-prebiotic combinations (synbiotics) to maximize host benefits.

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