Glycan Utilization and Cross-Feeding Activities by Bifidobacteria

Trends in Microbiology

CellPress REVIEWS

Special Issue: Broad Concepts in Microbiology

Review

Glycan Utilization and Cross-Feeding Activities by Bifidobacteria

Francesca Turroni,[1,2] Christian Milani,[1] Sabrina Duranti,[1] Jennifer Mahony,[3] Douwe van Sinderen,[3] and Marco Ventura[1,2,*]

Bifidobacteria represent one of the first colonizers of the mammalian gut, where such colonization is facilitated by their saccharolytic capabilities. Genomic analyses of bifidobacteria have revealed intriguing genetic strategies employed by these bacteria to access a variety of dietary and host-produced glycans. Bifidobacterial genome evolution therefore represents a fascinating example of how their chromosomes were molded to contain a large number of genes involved in carbohydrate metabolism. One of the reasons as to why bifidobacteria are such dominant and prevalent members of the (early) microbiota is that they may access glycans in the gut through mutualistic cross-feeding or resource-sharing activities, which is indicative of 'social behavior' among bifidobacterial strains.

Trends

Bifidobacteria represent key members of the gut microbiota in the early phases of life of animals that subject their offspring to parental care.

The first microbiota assemblage is believed to play pivotal roles in human health, both in infancy and at later stages of life.

Dietary and/or host-derived glycans represent a potent evolutionary force that has shaped the bifidobacterial pan-genome.

In silico and functional genomic analyses of bifidobacterial genomes revealed species-specific adaptation to a glycan-rich gut environment.

A key determinant of microbial dynamics in the gut microbiota results from nutrient competition and sharing.

Bifidobacteria play an important ecological role in shaping the gut microbiome.

Bifidobacteria exhibit social behavior through carbohydrate resource sharing in the gut.

Commensal Bifidobacteria

Bifidobacteria (see Glossary) are dominant key gut commensals of humans that colonize their host very quickly following birth [1], although their relative abundance decreases following weaning and with ageing. Bifidobacteria enjoy a wide ecological distribution, and can commonly be found in the gastrointestinal tract of many animals, including all (assessed) mammalian species, as well as the gut of certain insects and birds [1,2]. Interestingly, these apparently unrelated ecological origins may represent a common niche of the gastrointestinal tract from hosts that are social animals whose offspring enjoy parental care. Therefore, their ecological distribution is perhaps enabled by direct transmission of bifidobacterial cells from mother/care-provider to newborn. In fact, in humans, bifidobacterial colonization occurs immediately following delivery and is driven by maternal inheritance via vertical transmission from mother to baby [3,4]. Among the bifidobacterial species identified in primates (including humans) it is possible to distinguish bifidobacterial taxa that are typically found in adults, such as *Bifidobacterium adolescentis* and *Bifidobacterium catenulatum*, while others are much more commonly found in the gut of breast-fed infants such as *Bifidobacterium bifidum*, *Bifidobacterium breve*, and *Bifidobacterium longum* subsp. *infantis* [5]. Nevertheless, there does not seem to be a strict infant vs adult subdivision of bifidobacterial taxa. This makes sense in the context of vertical transfer of bifidobacterial species from mother to offspring, which includes adult-type members such as *B. adolescentis* [6,7].

Early events in bacterial inoculation/colonization of the mammalian gut represent an intriguing example of microbe–host coevolution, where members of the gut microbiota of adult mammals are specifically transmitted to their newborn. In this context, genomically identical bifidobacterial strains have been identified from stool samples of mother and child combinations, and the corresponding human milk samples are indicative of a **vertical transmission** route from the maternal gut to breast-fed infants [3,4,8,9]. This finding supports the notion that bacterial

[1]Laboratory of Probiogenomics, Department of Chemistry, Life Sciences and Environmental Sustainability, University of Parma, Parma, Italy
[2]Microbiome Research Hub, University of Parma, Parma, Italy
[3]APC Microbiome Institute and School of Microbiology, Bioscience Institute, National University of Ireland, Cork, Ireland

*Correspondence: marco.ventura@unipr.it (M. Ventura).

colonization of the infant gut depends on the mother's fecal/vaginal microbiota as well as on breast milk [10]. It has been proposed that bifidobacterial cells may reach the mammary gland of the mother through a systemic route and may be transmitted directly to breast-fed infants, though inversely, bifidobacteria may be introduced into human milk from the infant's oral cavity during suckling [11,12].

Milk-based transmission of bifidobacteria may be supported by their ability to utilize components of human milk such as human milk oligosaccharides (HMOs). Thus, it has been argued that the first microbiota of mammals encompasses gut commensals that are able to metabolize milk glycans, such as the bifidobacterial strains belonging to *B. longum* subsp. *infantis* and *B. bifidum* [13,14]. These pioneering bifidobacteria may be crucial to promote the establishment of subsequent bifidobacterial colonizers that are, by themselves, not able to directly access such complex, milk-derived carbohydrates. These bifidobacterial colonizers can utilize HMO-degradation products, including sialic acid and fucose, thanks to cross-feeding behavior [15–18].

The metabolic capabilities of many members of the human gut microbiota are skewed toward metabolism of mono-, di-, or/and oligo-saccharides, even though most dietary glycans reach the gut as polysaccharides. An interesting type of metabolic interaction of gut commensals is known as cross-feeding or syntrophy [19,20], whereby organisms that are capable of processing polysaccharides are able to sustain other members of a given community. Such a phenomenon may occur as a consequence of competition for the released carbohydrates, differential utilization of released components of polysaccharides, or further modification of fermentation products [21].

Bifidobacteria constitute up to 80% of the total complement of the gut microbiota in healthy, breast-fed infants [5,22]. Nevertheless, their relative abundance is considerably reduced after weaning and in the elderly [23]. Interestingly, bifidobacterial abundance in patients suffering from gastrointestinal diseases/disorders, such as diarrhea, colic, allergy, necrotizing enterocolitis, and obesity, is significantly reduced compared to healthy controls [23,24]. This suggests that (certain) bifidobacteria make an important contribution to **gut microbiota homeostasis**, which may be elicited through host–microbe interactions and/or their **cross-talk** with other members of the gut microbiota.

This review covers biological knowledge on the coevolution trajectory followed by bifidobacteria and their hosts in terms of genetic adaptation to the mammalian gut and co-operative behavior exhibited by these microorganisms in order to gain access to glycan resources available in this environment.

Genomics Explaining the Saccharolytic Features of Bifidobacteria

During the past 15 years, our understanding of bifidobacterial biology has significantly advanced due to a high number of scientific publications, highlights of which are, among others, the decoding of the first genome of *B. longum* subsp. *longum* NCC2705 [25] and the detailed reconstruction of the collated genetic arsenal of all known members of the *Bifidobacterium* genus, thus representing the **pan-genome** of this taxon [26]. Notably, the functional classification of the bifidobacterial pan-genome revealed that approximately 14% of the identified bifidobacterial genes encode enzymes involved in carbohydrate metabolism [26–28] (Figure 1). Comparative genome analyses based on the genetic arsenal of each of the currently known bifidobacterial (sub)species allowed the identification of a shared gene set, designated the bifidobacterial **core genome**, and of particular genes that are uniquely identified in a specific bifidobacterial taxon, and therefore represent Truly Unique Genes (TUGs)

Glossary

Bacterial cross-talk: instances in which one or more microbes affect the behavior of other microbes. It may be seen as metabolic communication between members of the gut microbiota.
Bifidobacteria: high G+C Gram-positive bacteria belonging to the phylum Actinobacteria representing common inhabitants of the gastrointestinal tract (GIT) of mammals, birds, and certain cold-blooded animals.
Core genome: the pool of genes that are shared by all strains of a particular bacterial taxonomic group, such as a species or a genus.
Glycobiome: the collective enzyme arsenal involved in carbohydrate uptake and metabolism.
Gut microbiota homeostasis: the situation where the gut microbiota composition remains in a stable, 'healthy' state.
Pan-genome: the full complement of genes of a bacterial species (or higher taxonomic group). The pan-genome includes the core genome (see above) and the dispensable genome containing genes present in only a subset of the examined taxonomic group.
Saccharolytic capabilities: the ability to retrieve energy from the metabolism of complex carbohydrates.
Vertical transmission: the ability of genetic material, including genomes of bacteria, viruses, and other organisms, to be passed on from mother to child (maternal inheritance).

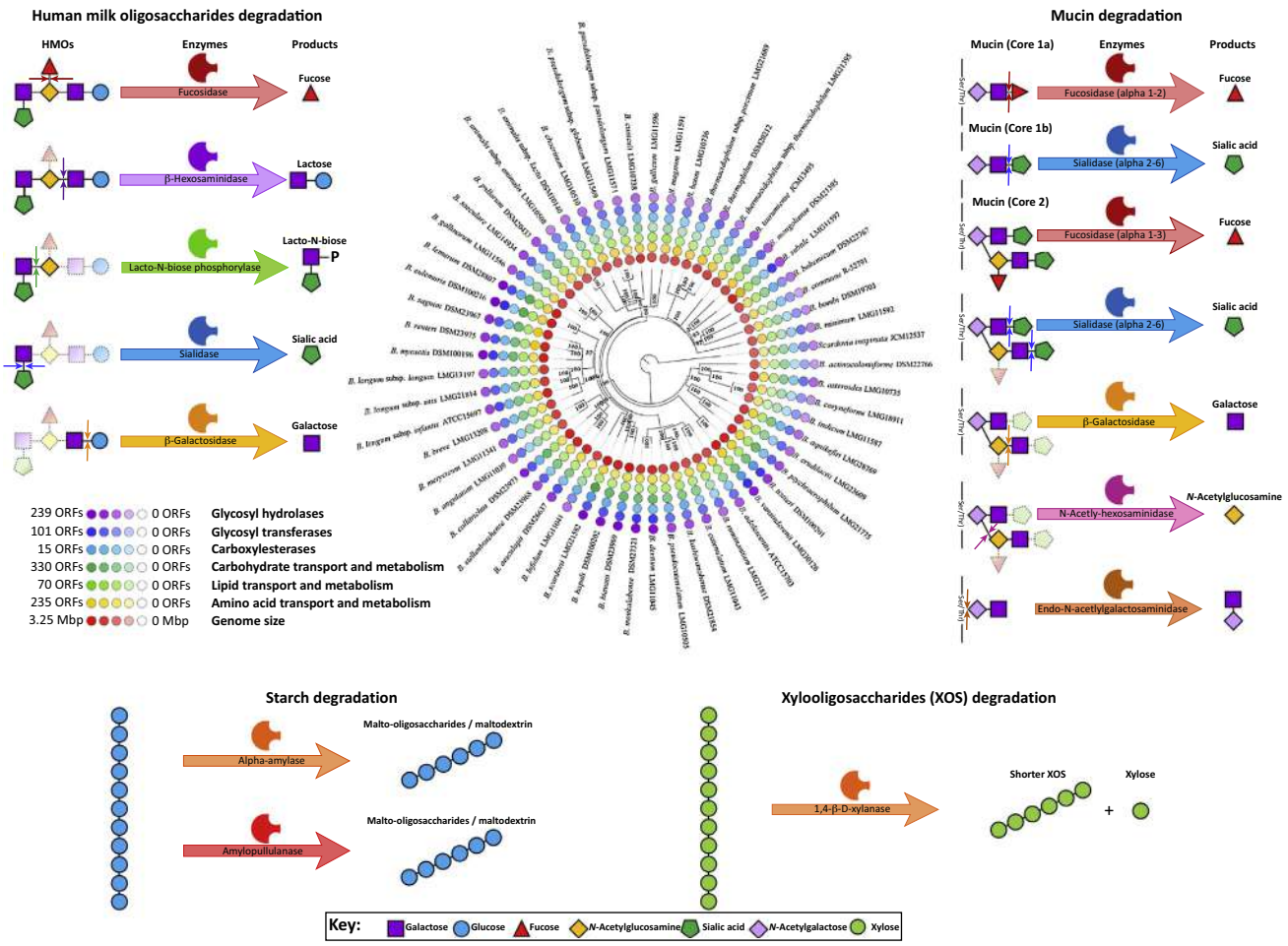

Figure 1. Glycobiome of the *Bifidobacterium* Genus. Phylogenetic reconstruction of bifidobacteria is reported as a super tree based on the 233 core genes identified for this genus. The outer circles illustrate the genome size as well as the number of open reading frames (ORFs) involved in amino acid transport and metabolism, lipid transport and metabolism, and carbohydrate transport and metabolism, or encoding carboxylesterases, glycosyl transferases, and glycosyl hydrolases. Moreover, schematic representations of pathways for the complete degradation of human milk oligosaccharides, starch, mucin, and xylooligosaccharides are shown.

[26]. Interestingly, the bifidobacterial core genome contains all genes of the bifid shunt, which is believed to support the ecological success of bifidobacteria due to its superior energy output compared to other carbohydrate fermentative pathways [29]. In addition, 15% of the identified bifidobacterial TUGs account for proteins involved in carbohydrate metabolism, such as glycosyl hydrolases (GHs), and in carbohydrate uptake [26]. Acquisition of GH-encoding genes is predicted to have occurred during the very early stages of bifidobacterial speciation (e.g., through horizontal gene transfer) [26,27]. Notably, members of GH3 and GH43 families, which are commonly associated with the degradation of plant polysaccharides, are among such early acquired GHs. Additionally, members of the large GH13 family, which encompasses α-amylases, are believed to have been acquired prior to the evolutionary split of bifidobacteria from other members of the Bifidobacteriaceae family [30]. Interestingly, they appear to have been lost in the genomes of bifidobacteria isolated from social insects [31], likely as a consequence of

adaptation to a glycan milieu rich in simple sugars such as those found in the hindgut of bees. In addition, many putatively acquired genes encode predicted carbohydrate uptake functions, such as ATP-binding cassette (ABC) transporters, phosphoenolpyruvate-phosphotransferase systems (PEP–PTS), and members of the major facilitator superfamily (MFS) [26,27].

Altogether, these data reinforce the assumption that the bifidobacterial genome content reflects glycan availability in the corresponding ecological niche, and that bifidobacterial evolution was guided by selective adaptation to establish versatile carbohydrate metabolism [2,28,32,33].

Gut microbiota members can broadly be divided into generalists and specialists according to their ability to utilize nutritional resources, including glycans [34]. Such different capabilities lead to varying ecological distributions, and may support a wide range of habitats for generalist players, such as members of the Bacteroides phylum, while allowing a narrow ecological distribution for specialists like members of the Firmicutes phylum [35]. However, when this approach is applied to other key gut commensals, such as bifidobacteria, one can identify both of these ecological strategies among members of the genus *Bifidobacterium* [27,28]. Dissection of the pan-genome of the *Bifidobacterium* genus, as based on the Carbohydrate-Active Enzymes (CAZy) system [36], has revealed that the bifidobacterial **glycobiome** represents one of the largest predicted enzyme arsenals for carbohydrate metabolism so far identified among known gut commensals. The bifidobacterial glycobiome consists of 3385 genes encoding predicted (or sometimes proven) carbohydrate-active enzymes, encompassing GHs, glycosyltransferases (GTs) and carbohydrate esterases (CEs), that are found across 57 GH, 13 GT, and seven CE families, respectively [26–28] (Figure 1). Notably, GH13 represents one of the most abundantly occurring GH families within the bifidobacterial glycobiome (Figure 1), targeting the breakdown of a plethora of complex glycans involving starch, glycogen, and related substrates such as amylose, amylopectin, pullulan, maltodextrin, and cyclomaltodextrin, as well as palatinose and turanose [37], representing all dominant carbohydrates found in the (modern) adult human diet. Nevertheless, it is worth mentioning that GH13 enzymes also occur at high prevalence in other members of the mammalian gut microbiota, where, apart from the hydrolytic functions described above, they are involved in intracellular α-glucan metabolism as associated with the synthesis and breakdown of the energy-storage polymer glycogen [38]. The high abundance of GH13, together with other plant-related GH families such as GH3, GH43, and GH51 in the glycobiomes of certain bifidobacterial taxa (e.g., *B. longum* subsp. *longum*, *B. adolescentis,* and *B. catenulatum*) suggests an adaptation of these bifidobacterial species to avegetarian or omnivorous diet,consistent with a more generalist ecological behavior. However, stachyose can only be partly degraded by members of this GH13 family.

By contrast, glycobiomes of certain bifidobacterial species, such as *B. bifidum* and *B. longum* subsp. *infantis*, are enriched in GH families that are essential for host-glycan degradation, such as those belonging to GH33 representing exo-sialidases, GH29, which encode fucosidases, and GH20, which include hexosaminidase and lacto-N-biosidase activities [17,27]. Such a GH content suggests that such bifidobacteria have adopted a very particular ecological specalization toward the infant gut or a mucosal environment. Other specialist bifidobacterial species are those isolated from social insects (e.g., *Bifidobacterium asteroides*, *Bifidobacterium coryneforme*, *Bifidobacterium indicum*, *Bifidobacterium actinocoloniforme*, *Bifidobacterium bohemicum*, and *Bifidobacterium bombi*), whose glycobiomes are enriched in GH families dedicated to the utilization of simple sugars, which are commonly identified in the diet of social insects [27,31]. The glycobiome of bifidobacteria isolated from social insects predicts the

presence of a metabolic pathway involved in the utilization of trehalose, which represents a typical glycan storage and blood-sugar of many insects, indicative of a genetic adaptation of these bifidobacterial species to a narrow ecological niche.

In addition, many identified GHs from the deduced bifidobacterial glycobiome (e.g., GH43 and GH51 members, annotated as α-l-arabinofuranosidases, as well as putative sialidases which typically belong to the GH33 family) are predicted to be secreted [7,26,27,39–41]. Their presumed extracellular activity may thus result in debranching and/or liberating monosaccharides or simple sugars in the gut environment, and such released carbohydrates may then be accessed by other members of the gut microbiota.

Glycan Utilization Profiles of Bifidobacteria and Their Genetic Adaptation to the (Human) Gut

A crucial metabolic contribution made by bifidobacteria to their hosts is the breakdown of diet-derived glycans (e.g., starch, maltodextrin, pullulan, [arabino]xylan and xylo-oligosaccharides) as well as carbohydrates contributed by the host and known as host-glycans such as glycoproteins (e.g., mucin) and HMOs, which match the **saccharolytic abilities** of certain *Bifidobacterium* species [27]. Notably, the metabolic activities exerted by bifidobacteria toward HMOs, which represent the first prebiotic compounds available at the very early stages of life in human beings, are pivotal in their establishment and persistence in the gut [42]. *In silico* analyses of the genomes of *B. longum* subsp. *infantis* ATCC15697 and *B. bifidum* PRL2010 has revealed how these two bifidobacterial species are able to utilize host-derived glycans (e.g., HMOs and mucin). In particular, the genome of *B. longum* subsp. *infantis* ATCC15697 encompasses a genetic locus containing genes that encode enzymes responsible for the breakdown of HMOs (e.g., fucosidase, sialidase, β-hexosaminidase, and β-galactosidase) and internalization of HMOs (e.g., extracellular solute binding proteins and permeases of ABC transporter systems) [13] (Figure 1). In addition, the chromosome of this strain contains a genetic locus involved in the metabolism of urea, which represents an important nitrogen source in human milk [13]. Remarkably, maternal genotypes that determine fucosylation patterns of HMOs play a role in the assemblage of the infant gut microbiota before weaning [43]. In this context, specific infant-associated bifidobacteria, such as *B. longum* subsp. *infantis* and *B. bifidum* species, can efficiently metabolize HMO components, such as lacto-N-tetraose [13,44], and consequently play a key role in the establishment of the first gut microbiota in the early stages of life.

Another host-produced glycan is mucin, which forms one of the main barriers that cover the gastrointestinal mucosa. The main glycan components of mucins are oligosaccharides composed of *N*-acetylglucosamine, *N*-acetylgalactosamine, fucose, and galactose moieties, and typically capped by sialic acid and/or sulfate groups [45]. The enzymatic repertoire to degrade (the glycan parts of) these glycoproteins requires specific GHs, such as the endo-α-*N*-acetylgalactosaminidase, which catalyzes hydrolysis of O-glycosidic α-linkages between galactosyl β-1-3 *N*-acetylgalactosamine and serine or threonine residues [46], and particular fucosidases, which liberate l-fucose from the oligosaccharide core of the mucin structure [47]. Additional enzymes needed for hydrolysis of mucin-derived glycans include *N*-acetyl-β-hexosaminidases, β-galactosidases, and sialidases [48]. Moreover, the core oligosaccharide structure of mucin is represented by galacto-N-tetraose, which is metabolized into galacto-N-biose (GNB) by a small number of human gut microbiota members, including specific bifidobacterial species [44]. GNB is then transported into the cell due to a dedicated ABC-type transporter, cleaved and phosphorylated by a galacto-N-biose phosphorylase, and finally utilized in the glycolytic and amino-sugar metabolic pathways [48].

Interestingly, just a small number of human gut microbiota members can directly access mucin as a carbon source. It has been demonstrated that *B. bifidum* and *Akkermansia muciniphila* strains are able to efficiently metabolize mucin [40,41,49,50]. In this context, *in silico* analyses coupled with functional genomic investigations of the chromosomal sequences of *B. bifidum* PRL2010 outlined the genetic arsenal responsible for mucin metabolism, which includes extracellular sialidases and fucosidases, a putative cell wall-anchored endo-α-*N*-acetylgalactosaminidase, *N*-acetyl-β-hexosaminidases, and β-galactosidases [41] (Figure 1). Notably, such a genetic repertoire is part of the unique core genome sequences of members of the *B. bifidum* species [39], suggesting the existence of an intriguing case of coevolution of a human gut commensal to the human intestine, where host-produced glycans act as a carbon and energy source for its establishment and survival within the human gut [41].

Another significant genetic display of bifidobacterial adaptation to the human gut is represented by the specific utilization of various complex glycans, including resistant starch, which is derived from the diet and which escapes host-directed digestion. The starch structure includes amylose and amylopectin moieties, with the former being a linear α-(1,4) glucose chain with a plant-specific degree of polymerization of 200–6000, and the latter representing short linear α-(1,4)-glucose linked chains with α-(1,6)-linked glucose side chains [51]. Common glycan derivatives of starch include maltodextrin and maltose [51]. Hydrolysis of starch and its derived saccharides is performed by saccharolytic gut commensals such as *Ruminococcus bromii* [52], *Bacteroides thetaiotaomicron* [53], and *Roseburia inulinivorans* [54], through the combined action of amylases and amylopullulanases. It has been established that bifidobacteria are nondominant members of the adult gut microbiota; nevertheless, their biological contribution to the metabolism of dietary glycans has been documented [55]. In this context, *in silico* analyses of genome sequences of *Bifidobacterium* (sub)species revealed that the presence of genes encoding the starch/starch-derivative-degrading enzymes mentioned above is not a conserved feature among human gut bifidobacterial strains but is largely confined to specific taxa and strains such as *B. adolescentis* [6,7,56] (Figure 1) and *B. breve* [57,58]. The deduced *B. adolescentis* glycobiome reveals that, when compared to other members of the *Bifidobacterium* genus, it contains a more expanded set of GH13 enzymes, which include predicted amylase, pullulanase, and cyclomaltodextrinase activities, thus explaining its superior growth performance on plant-derived carbohydrates [52] (Figure 2). The latter growth performance is correlated to the presence of an amylopullulanase containing two catalytic modules and associated carbohydrate-binding modules, which are crucial for efficient substrate catalysis and possible attachment of cells to starch granules [56]. Notably, the acquisition of these enzymes was predicted to have occurred during the early stages of *B. adolescentis* speciation, indicating that the preference for starch metabolism already existed in immediate *B. adolescentis* ancestors [7].

By contrast, the uptake system and the intracellular machinery required to metabolize alpha-(1,4)-malto-oligosaccharides and alpha-(1,6)-isomalto-oligosaccharides (GH13_31) was shown to be ubiquitously present in bifidobacteria [56].

Dietary fibres may encompass xylan and xylo-oligosaccharides, which can be accessed by members of the gut microbiota in different ways. Notably, bifidobacteria, in contrast to other enteric microorganisms, such as *Bacteroides* and *Roseburia*, exhibit limited hydrolytic capabilities toward xylan, a property that is reflected by the very rare occurrence of genes encoding GH10 xylanases in currently published bifidobacterial genomes [27,59]. By contrast, growth on xylo-oligosaccharides is relatively conserved among members of the *Bifidobacterium* genus [60]. In fact, bifidobacteria that are unable to grow on xylan may cross-feed efficiently on (the

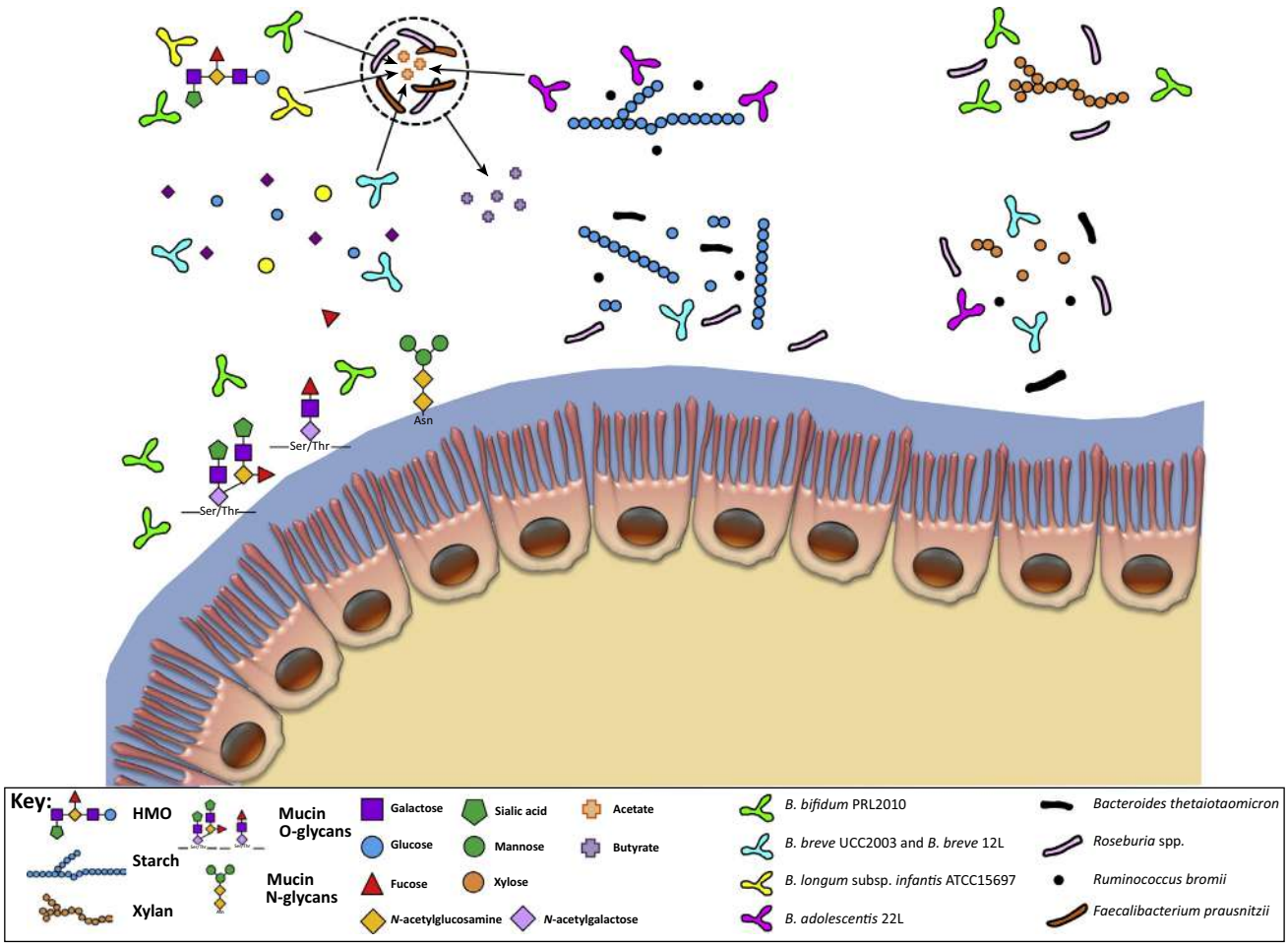

Figure 2. Examples of Glycan Degradation Activities of Bifidobacteria in the Mammalian Gut. Bifidobacterial activities toward different complex carbohydrates produced by the host, that is, mucin (O-glycans and N-glycans) and HMOs, or derived from the diet (e.g., starch and xylan), are presented. Hydrolysis of complex sugars by specific bifidobacterial strains produces simple glycans that are utilized directly by the same bifidobacterial cells and/or are metabolized by other bacterial cells belonging to different strains/species through cross-feeding or resource-sharing activities.

derived oligomers of) this substrate, when cultivated with *Bacteroides ovatus*, owing to the extracellular xylan-degrading activity of *B. ovatus*, thereby allowing efficient uptake of the produced xylo-oligosaccharides by a dedicated ABC transporter encoded by many bifidobacterial species [61].

Among the bifidobacterial communities that reside in the gut of infants or adults, certain bifidobacterial species, such as *B. breve*, possess assimilation capabilities for a wide range of carbohydrates, which include both dietary and host-derived glycans [37,58,62–64].

The pan-genome of *B. breve* indeed highlights extensive, but sometimes variable, genetic capabilities with regard to the metabolism of carbohydrates, including, among others, ribose and sucrose, as well as the plant-derived polysaccharides starch, galactan, and cellodextrin [65].

Despite its extensive carbohydrate-processing abilities, *B. breve* is not able to directly access mucin or HMOs as carbon sources, though it can utilize host-derived mono/oligosaccharides once they become available through hydrolytic activities of other (bifido)bacteria present in the human gut microbiota (see below in this review) [15–17,27,66]. In this context, it has been observed that, within *B. breve* species, the growth on total HMOs is moderate, with good growth on LNT and lacto-N-neotetraose [67]. Similarly, other bifidobacteria, for example, *B. pseudocatenulatum* and *Bifidobacterium kashiwanohense*, display an ability to partially utilize HMOs [67–69], and thus it is plausible that they can grow on this host glycan by cross-feeding activities that are dependent on other bifidobacterial HMO utilizers.

Functional Contributions by Bifidobacteria to the Human Gut Glycobiome

Analysis of the human gut glycobiome in infants and adults has highlighted a pivotal contribution of bifidobacteria. Interestingly, despite the relatively low abundance of bifidobacteria in the adult human gut, their functional contribution to the human gut microbiome may be important in terms of expanding the overall glycobiome of the large intestine, thereby influencing the overall gut physiology [27]. Notably, a recent human feeding trial, involving the *Bifidobacterium longum* subsp. *longum* AH1206 strain, revealed that gut colonization and persistence of this strain in certain individuals is linked to depletion of certain genes involved in carbohydrate metabolism from the gut microbiome in such individuals, thus providing a niche for AH1206 which was shown to encode these depleted metabolic abilities [70]. Among the most frequently represented GHs encoded by bifidobacteria in the adult gut glycobiome, it is worth mentioning the extensive repertoire of GHs involved in the breakdown of complex plant carbohydrates, such as GH3, GH13, GH43, GH51, and GH77 [27]. In the gut glycobiomes of infants, by contrast, bifidobacteria are significantly contributing to the pool of GHs responsible for catabolism of milk-related carbohydrates such as lactose and HMOs, and/or involved in mucin degradation [27].

Such findings are further supported by metatranscriptome data, which revealed pronounced transcription of those bifidobacterial GH gene families predicted to be involved in complex plant carbohydrates, as well as HMO and mucin degradation [27], thus clearly supporting the important functional roles exploited by bifidobacteria in the breakdown of various glycans in both the infant and adult human gut.

The impact of bifidobacteria on the metabolism of dietary and host-derived glycans is also pivotal for the establishment and reinforcement of trophic relationships between members of the gut microbiota [18,70,71].

Simple Altruistic/Egocentric Activities of Various Bifidobacteria

Bifidobacterial populations establish several trophic interactions with each other as well as with other members of the gut microbiota, leading to competition for or co-operative sharing of nutrients through typical cross-feeding behaviour. In this context, microbe–microbe interactions can either positively or negatively influence the fitness of affected organisms [72] by the release of molecules in the environment [20,73]. Cross-feeding strategies developed by enteric microorganisms promote an expansion of their glycan acquisition capabilities, thus enhancing the ecological fitness of a specific proportion of, or even the overall, gut microbiota [27]. Cross-feeding activities in the human gut are facilitated by primary microbial saccharolytic microorganisms such as bifidobacteria, which, through partial extracellular breakdown of specific complex glycans (e.g., host-produced glycans), liberate monosaccharides and/or oligosaccharides in the gut environment that become accessible to other microbial gut inhabitants [74]

(Figure 2). This role of a primary degrader applies just to a narrow taxonomic group and certainly not to all members of the *Bifidobacterium* genus. The subsequent fermentative metabolism of these carbohydrates also produces metabolic end products, such as acetate and lactate, which, in turn, may act as substrates for other microbial gut colonizers, in particular the butyrate-producing enteric bacteria [75–79].

Specific cases of cross-feeding activities among members of bifidobacterial communities have experimentally been demonstrated to occur between the infant-type bifidobacterial strains *B. bifidum* PRL2010 and *B. breve* UCC2003, when these microorganisms are grown on sialyl lactose as the only carbon source [15,16]. Recently, additional cross-feeding activities have been observed between a set of bifidobacterial strains (*B. bifidum* PRL2010, *B. breve* 12L, *B. adolescentis* 22L, and *B. thermophilum* JCM7017) when cultivated on plant-derived glycans such as starch and xylan [17]. Cocultivation experiments, coupled with transcriptomic and metabolomic assays, highlighted that the concurrent presence of the above-mentioned bifidobacterial strains provokes an increase in the metabolic activity of *B. bifidum* PRL2010. This indicates that PRL2010 cells take advantage of the concomitant presence of (and glycolytic activities produced by) other bifidobacterial strains.

Furthermore, *in vivo* trials involving conventional mice receiving bifidobacterial cells belonging to *B. bifidum* PRL2010, *B. longum* subsp. *infantis* ATCC15697, *B. adolescentis* 22L, and *B. breve* 12L, further confirmed the existence of cross-feeding activities between various bifidobacterial strains in the mammalian gut [18]. Notably, in this study, transcriptomic experiments coupled with metagenomic analyses of single, dual, or multiple associations of bifidobacterial strains uncovered cross-feeding activities, which showed a clear expansion of the murine gut glycobiome toward its enzymatic potential related to the breakdown of complex carbohydrates involving plant-derived carbohydrates such as xylo-oligosaccharides, arabinoxylan, starch, and host-glycan substrates. Moreover, these *in vivo* assays highlighted differential metabolic strategies exerted by various bifidobacterial strains toward carbohydrates, exemplified by a 'selfish' behavior displayed by *B. longum* subsp. *infantis* ATCC15697 as it internalizes HMOs prior to degradation, thereby eliminating the possibility of resource sharing by other enteric bacteria. However, *B. longum* subsp. *infantis* will still release metabolites such as acetate and lactate, which could be used by other gut microorganisms (see above). By contrast, *B. bifidum* PRL2010 actively participated in the extracellular catabolism of host glycans and thus in the release of simple sugars that can, in turn, be utilized by other members of the (bifido)bacterial community [18]. Such a scenario has also been observed in other *B. bifidum* strains, thus reinforcing a specific ecological role exploited by the members of this bifidobacterial taxon [80].

Recently, other bifidobacterial cross-feeding strategies toward utilization of fucose have been discovered, involving various *B. bifidum* as well as *B. kashiwahonense* and *B. breve* or *B. longum* subsp. *infantis* strains, which result in the formation of 1,2-propandiol [69,81,82].

Cross-feeding activities of bifidobacteria have been observed to be also directed to other gut microorganisms. In this context, the metabolic cross-feeding between *B. adolescentis* and lactate-utilizing, butyrate-producing bacteria such as *Eubacterium hallii* and *Anaerostipes caccae* have been described [83].

Notably, the more common role of bifidobacteria is cross feeding on simpler or solubilized glycans/oligosaccharides. Nevertheless, in contrast to other gut microorganisms known to exploit similar cross-feeding activities very little is known about the mechanisms mediating such phenomena and the importance of glycan transporters [84].

Altogether, these findings corroborate the notion that members of the bifidobacterial communities participate in social behavior through glycan resource sharing in the gut ecosystem, thereby forging trophic relationships between gut microorganisms in mammals.

Concluding Remarks

Bifidobacteria, like other gut saccharolytic microorganisms, rely heavily on the availability of glycans. These macromolecules have driven the evolution of bifidobacteria and greatly impacted the genome content of these microorganisms. In this context, one may argue that cross-feeding behavior elicited by bifidobacteria in their natural ecological niche has been the result of a coevolutionary trajectory followed by the different members of the bifidobacterial communities. This has resulted in co-operative behavior by bifidobacteria so as to gain access to glycan in the mammalian gut and in extensive co-occurrence rather than coexclusion trends of many of the currently known bifidobacterial species [1]. Bifidobacterial interactions result in metabolic dependencies, which may also be the reason for difficulties encountered when trying to isolate/cultivate (bifido)bacteria under laboratory conditions, thereby explaining why many gut bacteria are still considered 'unculturable'. Altogether, these data highlight the importance of understanding the ecology and evolution of metabolic interactions within natural microbial communities in order to gain insights into the biology of any microbe (see Outstanding Questions).

Disclaimer Statement

The authors declare that they have no competing interests.

Acknowledgments

This work was funded by the EU Joint Programming Initiative – A Healthy Diet for a Healthy Life (JPI HDHL, http://www.healthydietforhealthylife.eu/) and the MIUR to MV. We thank GenProbio srl for financial support of the Laboratory of Probiogenomics. DvS is a member of The APC Microbiome Institute funded by Science Foundation Ireland (SFI), through the Irish Government's National Development Plan (Grant number SFI/12/RC/2273). JM is supported by a Starting Investigator Research Grant (SIRG) (Ref. No. 15/SIRG/3430) funded by Science Foundation Ireland (SFI). Part of this research is conducted using the High Performance Computing (HPC) facility of the University of Parma.

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Outstanding Questions

How do bifidobacteria exchange glycan metabolites within microbial communities? Are physical structures produced by bifidobacterial cells important in the exchange of these metabolites?

Can bifidobacteria regulate their cross-feeding activities?

How do different genetic transmission modes, that is, vertical vs horizontal, involving genes encoding carbohydrate active enzymes, occur in bifidobacterial genomes? Are there mechanisms that may discipline non-cooperating bifidobacterial partners?

How evolutionarily stable are the cooperative systems identified in bifidobacteria to access to glycans? If bifidobacterial cells invest in the production of a catabolic enzyme that helps other members of the microbial community, what prevents these bifidobacterial cells from being outcompeted by the other cells that consume the hydrolytic products without producing the enzyme?

Can bifidobacteria regulate the production of glycan metabolites that are being exchanged?

How feasible will be the use of cross-feeding activities in future culturomics approaches to gain access to the so far 'unculturable' fraction of the human gut microbiota?

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