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MINIREVIEW

Genomics of the Genus *Bifidobacterium* Reveals Species-Specific Adaptation to the Glycan-Rich Gut Environment

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Bifidobacteria represent one of the dominant microbial groups that occur in the gut of various animals, being particularly prevalent during the suckling period of humans and other mammals. Their ability to compete with other gut bacteria is largely attributed to their saccharolytic features. Comparative and functional genomic as well as transcriptomic analyses have revealed the genetic background that underpins the overall saccharolytic phenotype for each of the 47 bifidobacterial (sub)species representing the genus *Bifidobacterium*, while also generating insightful information regarding carbohydrate resource sharing and cross-feeding among bifidobacteria. The abundance of bifidobacterial saccharolytic features in human microbiomes supports the notion that metabolic accessibility to dietary and/or host-derived glycans is a potent evolutionary force that has shaped the bifidobacterial genome.

The ecological relevance of bifidobacteria was immediately obvious when they were first isolated from stool samples of a breast-fed infant at the beginning of the last century (1). Members of the *Bifidobacterium* genus are Gram-positive bacteria that belong to the *Actinobacteria* phylum, and, together with the genera *Aeriscardovia*, *Alloscardovia*, *Gardnerella*, *Parascardovia*, and *Scardovia*, form the *Bifidobacteriaceae* family (2). The particular ecological role of the *Bifidobacterium* genus has been highlighted in recent years due to the identification of 47 different taxa, mostly isolated from the gut of social animals, which encompass mammals, birds, and insects (3), whose offspring are dependent on parental care. This appears to be a common ecological trademark of this bacterial genus and a feature that distinguishes it from other gut commensals like *Bacteroides* or *Lactobacillus*. This ecological habitat implies a special route of colonization, which is known as vertical transmission from mother to offspring and which in recent times has gained considerable scientific interest (4). In this context, it is widely accepted that the mammalian fetus develops in an essentially sterile environment within the amnion and that microbial colonization of the fetus commences as soon as the amnion breaks prior to delivery of the baby through the birth canal (4). Bifidobacteria are among the first bacterial colonizers of the human gut, and several (sub)species of this genus are genetically adapted to utilize the nourishment of infants through the metabolism of particular glycans present in human milk (5). Nevertheless, human milk not only represents an important reservoir of glycans that act as bifidogenic factors to specifically support growth of particular bifidobacteria but also acts as a repository of (bifido)bacteria for vertical transmission from mother to infant. This notion is corroborated by the isolation of bifidobacteria directly from human milk (6–8), although it is not clear how bifidobacteria reach this human bodily fluid (9). Similarly, bifidobacteria have been shown to be transferred from the gut lumen to tumors (and organs) by means of a route that is not fully understood (10). Only very recently, a study based on analysis of the gut microbiota of mothers and corresponding children by a combination of amplicon-based profiling and shotgun metagenomics demonstrated that mother and child share particular bifidobacterial strains, belonging to *Bifidobacterium breve* and *Bifidobacterium longum* subsp. *longum*, which is thus indicative of vertical transmission (11, 12). Mode of delivery (i.e., vaginally delivered versus delivered by cesarean section) and type of nutrition (i.e., breast-fed versus bottle-fed) are considered to be important factors that provide differential colonization opportunities, thereby impacting the composition of the neonatal gut microbiota, including the colonization level and species composition of bifidobacteria (13, 14).

As mentioned above, bifidobacteria have been reported to account on average for ~80% of the total complement of the gut microbiota in breast-feeding infants (15). However, their relative abundance significantly decreases after weaning, although overall numbers of bifidobacteria are only moderately reduced (16, 17). Notably, individuals suffering from gastrointestinal diseases have been shown to display a marked reduction in bifidobacteria compared to healthy controls (16, 17). The latter finding suggests that bifidobacteria play a role in the establishment/maintenance of gut homeostasis through host-microbe interactions and/or their direct interplay with other members of the gut microbiota.

The aim of this review is to shed light on the saccharolytic behavior of bifidobacteria and how bifidobacterial carbohydrate metabolism influences the overall glycobiome of the gut microbiota and host and contributes to trophic relationships between members of gut microbiota.

Genomics of bifidobacteria. The first fully decoded bifidobacterial genome was that of the human gut commensal *Bifidobacterium longum* subsp. *longum* NCC2705 (18). Since then, additional

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bifidobacterial strains have had their genomes sequenced, such as the adult human fecal isolate *Bifidobacterium longum* subsp. *longum* DJO10 (19) and the infant fecal isolates *Bifidobacterium bifidum* PRL2010 (20), *Bifidobacterium breve* UCC2003 (21), and *Bifidobacterium longum* subsp. *infantis* ATCC 15697 (5). Other bifidobacterial genomes that have been fully decoded include the human oral cavity isolate *Bifidobacterium dentium* Bd1 (22), as well as many strains belonging to the *Bifidobacterium animalis* subsp. *lactis* taxon, which due to their purported health-promoting activities have attracted a lot of commercial interest (23–25). Until 2014, genome sequences of only 10 of the 47 (sub)species assigned to the *Bifidobacterium* genus were available. Since then, all type strains of the currently recognized (sub)species belonging to this genus have been genomically decoded, thereby representing a genomic encyclopedia for the exploration of genetic variability within the genus *Bifidobacterium* (26). After the reclassification of *Bifidobacterium stercoris* as a junior synonym of *Bifidobacterium adolescentis* (27), the current number of (sub)species assigned to the genus *Bifidobacterium* is 47 (for which chromosomal sequences are available). Thus, all phylogenetic analyses that have recently been described in the study by Lugli et al. (28) have been reanalyzed in order to take the new *Bifidobacterium* genus layout into account. Characterization of the overall genetic content of members of the *Bifidobacterium* genus revealed genome sizes that ranged from 1.73 (*Bifidobacterium indicum*) to 3.25 Mb (*Bifidobacterium biavatii*), corresponding to 1,352 and 2,557 predicted protein-encoding open reading frames, respectively. Considering the close phylogenetic relationship between bifidobacteria (29), this substantial genomic size difference is reminiscent of an evolutionary pathway that has involved many gene loss and/or acquisition events. Functional classification of the overall genetic arsenal of the *Bifidobacterium* genus, representing the pan-genome of this taxon, revealed that 13.7% of the identified bifidobacterial genes encode enzymes involved in carbohydrate metabolism, which is higher than the percentage of such genes of many analyzed gut commensals (26). Among this large number of genes involved in the utilization of glycans, there is a notable presence of a genetic subset that is shared among all currently described 47 bifidobacterial (sub)species, thus being part of the bifidobacterial core genome, and of particular genes that are uniquely found in a specific taxon, and thus form part of the "truly unique genes" (TUGs) (26). Of particular note, among the bifidobacterial core genomic coding sequences, it is worth mentioning those genes that encode the enzymes that make up the bifid shunt (26). It is recognized that the evolutionary success of bifidobacteria may have been due to the fact that this particular metabolic pathway allows the generation of more ATP (per mole of glucose) than the other carbohydrate fermentative pathways, such as glycolysis or the pentose phosphate pathway (30). The evolutionary success of bifidobacteria is further compounded by the fact that they have specialized to metabolize either a very specific set or sometimes a broad range of dietary and/or host-derived glycans (20, 31, 32). Regarding the identified TUGs of the bifidobacterial pan-genome, it is estimated that 14.64% of these genes are encompassing proteins involved in carbohydrate metabolism, including glycosyl hydrolases (GHs) and proteins involved in carbohydrate uptake.

These data are a genetic reflection of the metabolic commitment of bifidobacteria to a saccharolytic lifestyle, which is a common genetic feature of many bacteria that make up the human gut microbiota (33).

Phylogenomics of the *Bifidobacterium* genus. A widely recognized approach applied in modern microbial taxonomy is DNA sequencing followed by comparison of 16S rRNA gene sequences (34). However, a major limitation of this method is that there are cases in which two taxa belong to distant bacterial groups, yet show high identity levels of their 16S rRNA gene sequences (35, 36). Recently, bifidobacterial taxonomy has benefited from the use of a multilocus or multigene approach based on alternative molecular markers such as *clpC*, *dnaB*, *dnaG*, *dnaJ1*, *purF*, *rpoC*, and/or *xfp* (29). Such a multigene approach allows a high level of discriminatory resolution between closely related bifidobacterial taxa and provides a robust means to infer phylogenetic relationships among members of the *Bifidobacterium* genus (29). In bacterial taxonomy, thanks to the availability of a growing number of complete genome sequences, it has become possible to reconstruct phylogenetic relationships between taxa on the basis of a much larger set of sequence data per taxon, thus allowing a more reliable and representative inference of the tree of life. In this context, comparative genomic analyses involving the chromosomal sequences from each of the 47 bifidobacterial type strains resulted in the identification of 18,260 *Bifidobacterium*-specific clusters of orthologous genes (BifCOGs), which constitute the pan-genome of the genus *Bifidobacterium* (see below). Notably, analysis of the predicted BifCOGs allowed the identification of 459 COGs that were shown to be shared between all these genomes, thus representing the core of bifidobacterial genome coding sequences (core BifCOGs), which can be employed as alternative molecular markers to the 16S rRNA gene sequences (28). A concatenated protein sequence encompassing the protein products of 413 core genes (where these genes had a single representative for each bifidobacterial genome) was used to build a *Bifidobacterium* supertree, which was shown to be superior in discriminatory power and robustness to a corresponding 16S rRNA gene-based tree (Fig. 1) (28). Such a phylogenomic approach has also been successfully employed for the delineation of other genera, such as *Lactobacillus* and *Streptococcus* (37, 38). This *Bifidobacterium* supertree highlights the evolutionary positioning of all 47 bifidobacterial taxa and reveals the existence of seven phylogenetic groups within the genus, in contrast to the previously identified six groups (29), due to the existence of a particular *B. bifidum* phylogenetic cluster (28). When a similar analysis was performed by also including representatives of the other genera of the family of the *Bifidobacteriaceae*, bifidobacteria were shown to fit in the deepest branch of the resulting phylogenetic tree, clearly separating them from other genera within this family (26). Notably, the *Bifidobacterium asteroides* phylogenetic group is positioned closest to the root in this family-based supertree, therefore suggesting that members of this group most closely resemble the evolutionary ancestor of the *Bifidobacterium* genus, as had been noted previously (39).

Evolutionary development of bifidobacteria. *In silico* analyses of the bifidobacterial genomes allowed the reconstruction of the genetic evolution of the (sub)species of this genus (26, 40). These analyses predict that the chromosome of the ancestor of the genus *Bifidobacterium* consisted of 967 COGs. This is just 196 COGs less than the number of COGs harbored by the *B. indicum* chromosome and 1,062 COGs less than the number in the *B. biavatii* genome, representing the smallest and largest genome, respectively (26). Thus, the evolutionary development of currently known bifidobacterial taxa appears to have involved a relatively small number of ancestral gene loss occurrences but a substantial

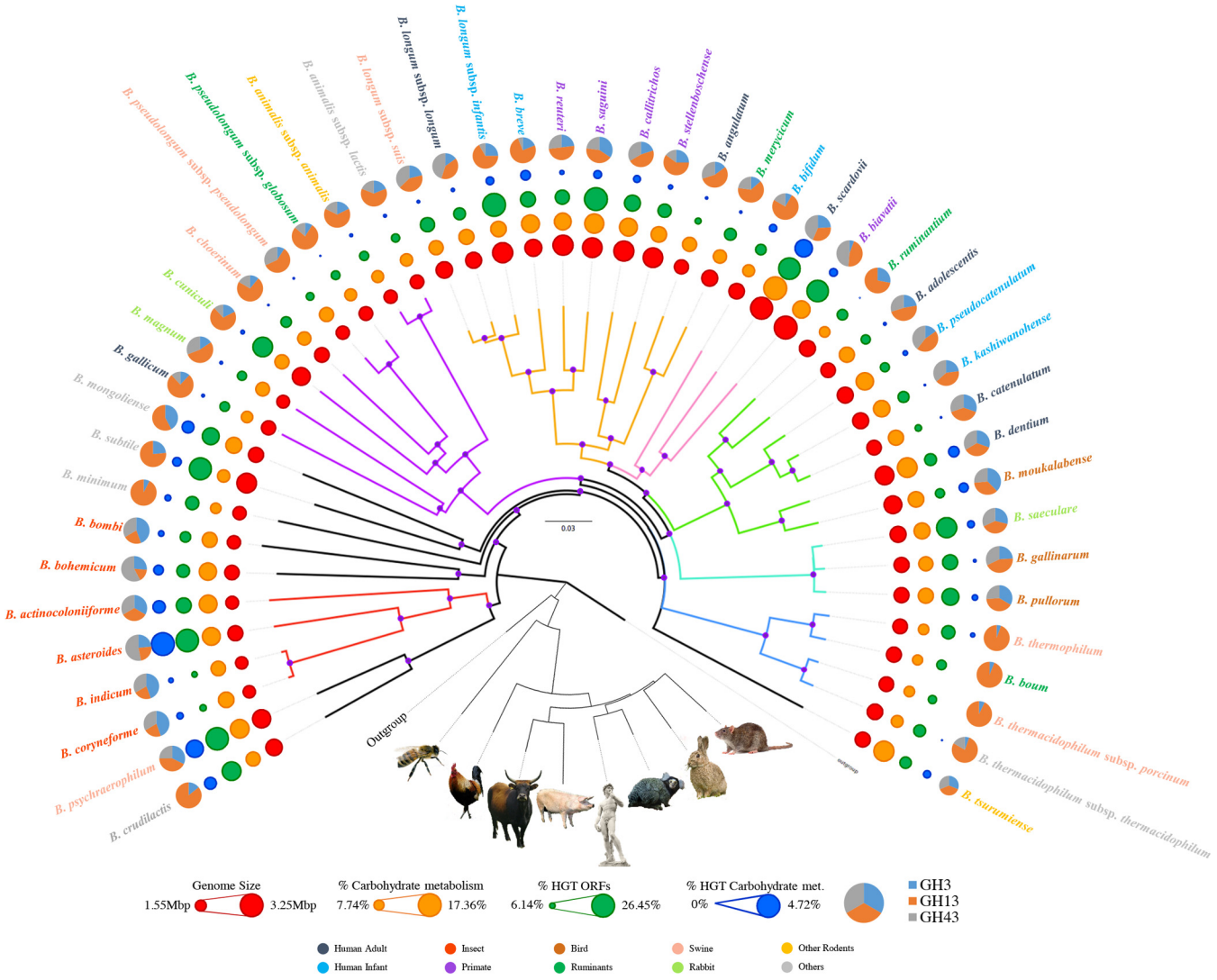

FIG 1 Phylogenomic overview of the genus *Bifidobacterium*. A supertree based on the alignment of 413 core COGs (with a single representative identified for each bifidobacterial genome) was constructed in order to obtain a robust phylogenetic reconstruction. Phylogenetic clusters are highlighted with branches of the same color, and nodes with bootstrap values higher than 70% are marked with a purple dot. Circles surrounding the tree represent the approximate genome sizes (in red), relative percentage of genes predicted to be involved in carbohydrate metabolism and transport (in orange), relative percentage of genes predicted to have undergone horizontal gene transfer (in green), and relative percentage of genes predicted to have been subject to horizontal gene transfer or to be involved in carbohydrate metabolism and transport (in blue). The outermost layer represents the proportion of GH families (i.e., GH3, GH13, and GH43). *E. coli*, *Escherichia coli*; met., metabolism; ORFs, open reading frames. Bifidobacterial species names are colored based on their ecological origin. In addition, the tree in the lower part of the image represents the phylogeny of the host species from which bifidobacteria had been isolated. This tree was constructed with the Superfamily database and software (85).

number of gene acquisition events (Fig. 2). In contrast, the genomes of other examined gut commensals, such as those of lactic acid bacteria, are believed to have undergone extensive genomic simplification (41).

In bacterial genomes, gene acquisition events that occur in the course of evolution are expected to facilitate adaptation to a novel ecological niche or to increase competitiveness in the existing ecological niche of the (micro)organism (29, 42). Investigation of gene families that are predicted to have been acquired by bifidobacterial genomes suggests that adaptation to an environment rich in complex glycans, like that of the animal gut, represented the main driving force responsible for speciation among members of the genus *Bifidobacterium*.

Genome-based analyses allowed a predicted reconstruction of gene acquisition as well as gene loss events that occurred in the course of evolution of bifidobacteria. In this context, eight COGs, including members of GH3 and GH43 families (GHs associated with the degradation of plant polysaccharides), were shown to have been acquired early in bifidobacterial speciation. In contrast, eight COGs encompassing members of the large GH13 family, representing α-amylases, were predicted to have been acquired during the evolution of the *Bifidobacteriaceae* family and prior to the acquisition of the eight aforementioned GH3 and GH43 members (Fig. 3). Moreover, several putatively acquired genes were identified with predicted carbohydrate uptake functions, including those that belong to the ATP-binding cassette (ABC), phos-

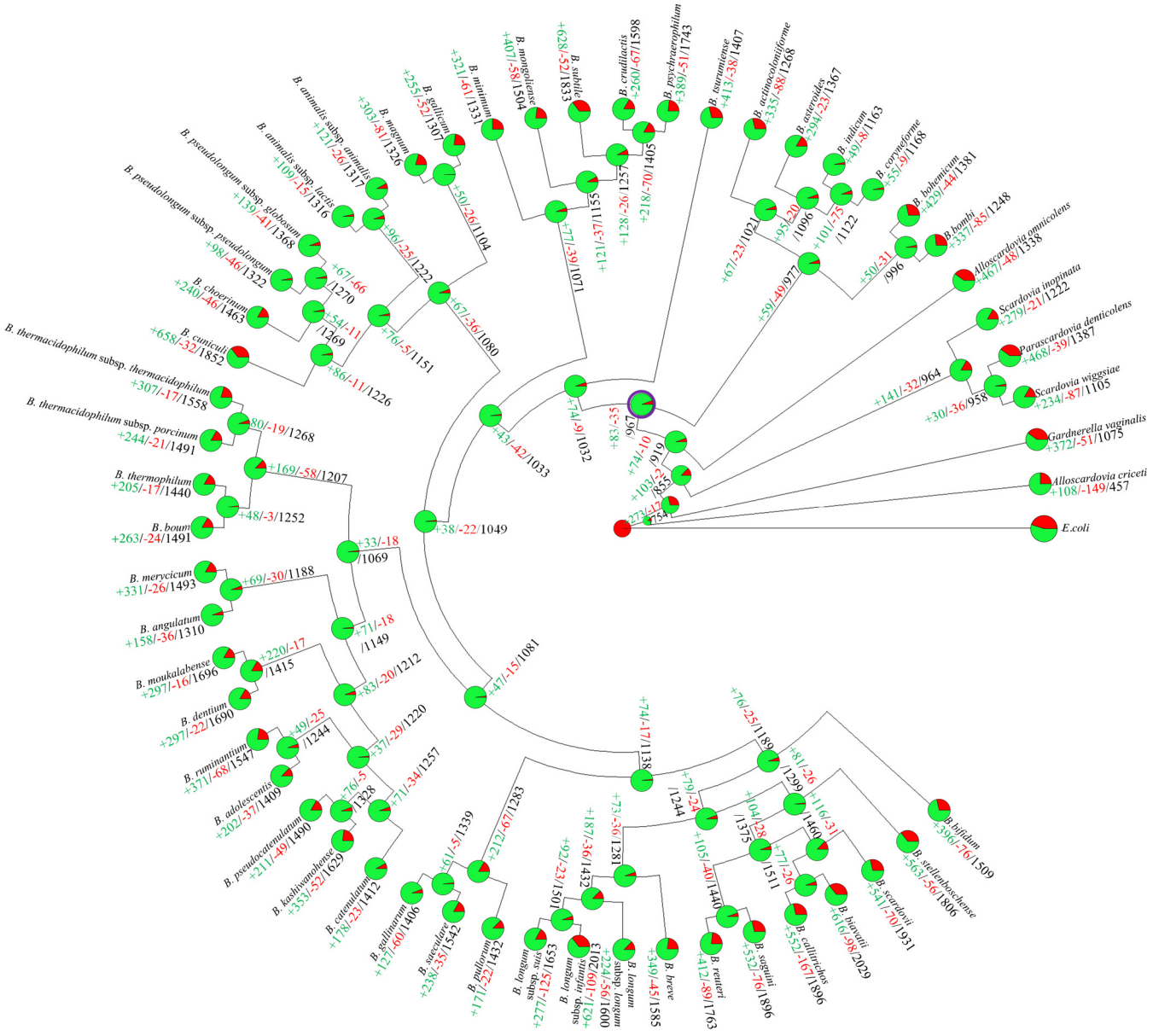

FIG 2 Gene gain and loss events in a reconstruction of data representing the family *Bifidobacteriaceae*. A tree was constructed based on information regarding the presence or absence of COGs for the whole *Bifidobacteriaceae* pan-genome. Each node is represented by a pie diagram showing the acquired COGs (in red) and the COGs derived from the previous node (in green). Furthermore, additional information is displayed at each node as follows: number of acquired genes, number of lost genes, and total number of COGs. The predicted *Bifidobacterium* ancestor is highlighted with a thick purple circle surrounding the pie diagram.

phoenolpyruvate-phosphotransferase system (PEP-PTS), and major facilitator superfamily (MFS) families of transporters (26).

Even if gene gain events represent the key driving force for bifidobacterial genome evolution, gene decay as well as metabolic simplification may still represent another crucial trend for niche-specific adaptation. *In silico* analyses of bifidobacteria revealed many gene loss events, most of which affect genes encoding biosynthetic enzymes, presumably reflecting analogous environmental pressures. Concerning GHs, most GH13 family members, encompassing α-amylases, appear to have undergone a gene decay process in the genomes of the clade encompassing bifidobacteria isolated from social insects (e.g., *B. asteroides*, *Bifidobacterium actinocoloniiforme*, *B. indicum*, *Bifidobacterim coryneforme*, *Bifidobacterium bombi*, and *Bifidobacterium bohemicum*), possibly as a consequence of a dietary modification of their host or, alternatively, as a consequence of adaptation to a new host with a different diet.

Notably, bifidobacterial species isolated from social insects are predicted to possess a complete trehalose degradation IV pathway, which is absent in the majority of the other bifidobacterial taxa (43). Thus, this may reflect a genetic adaptation of bifidobacteria to the insect gut since trehalose is used as carbohydrate storage and blood sugar by many insects, including the honeybee (43).

A large proportion of the predicted gene acquisition events are believed to be due to horizontal gene transfer (HGT) from other

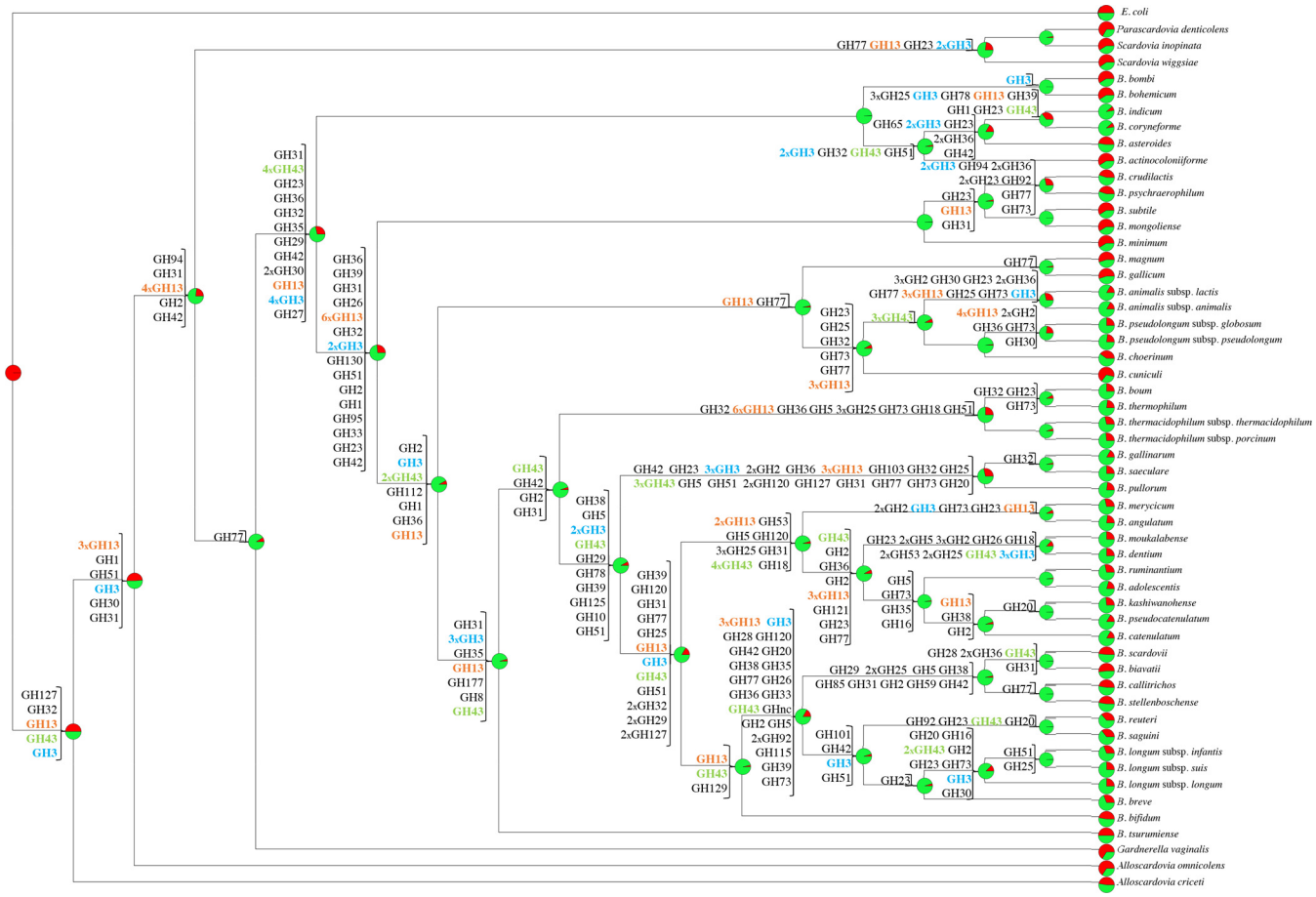

FIG 3 Reconstruction of gene gain and loss events regarding genes encoding members of the GH3, GH13, and GH43 families in the family *Bifidobacteriaceae*. A tree was constructed using information related to the presence or absence of COGs for the whole *Bifidobacteriaceae* pan-genome. For each node, a pie diagram shows the acquired COGs (in red) and the COGs derived from the previous node (in green). Furthermore, for each node the number of GH family members acquired is reported. (Gene decay events were omitted to allow readability of the figure.) Numbers are indicated when multiple COGs of the same GH family were acquired: otherwise only one COG was gained. GH3, GH13, and GH43 are colored in blue, orange, and green, respectively.

bacteria. The donors of these putative alien genes appear to have preferentially originated from other members of the *Actinobacteria* class (28.5%), followed by *Bacillus* (11.7%), *Gammaproteobacteria* (8.7%), *Clostridia* (8.7%), or *Alphaproteobacteria* (5.9%) (26). Importantly and perhaps not unsurprisingly, members of these predicted bacterial donors are also commonly found in the gut environment (44).

The glycobiome of bifidobacteria. Classification according to the Carbohydrate Active Enzymes (CAZy) system (45) indicates that the pan-genome of the *Bifidobacterium* genus encompasses one of the largest predicted glycobiomes among known gut commensals, consisting of 3,385 genes that encode putative carbohydrate-active enzymes, including glycosyl hydrolases (GHs), glycosyl transferases (GTs), and carbohydrate esterases (CEs), which are distributed across 57 GH, 13 GT, and 7 CE families (Fig. 4). In contrast, no polysaccharide lyase (PL)-encoding genes were found in the pan-genome of the *Bifidobacterium* genus (43). GH13 represents the most dominant GH family identified in the glycobiome of the genus *Bifidobacterium*. Enzymes of this family are characterized by their common catalytic activities of hydrolysis of a wide range of complex carbohydrates, such as starch, glycogen, and related substrates (e.g., amylose, amylopectin, pullulan, maltodextrin, and cyclomaltodextrin), as well as palatinose, stachyose, raffinose, and melibiose (32), which represent dominant glycans found in the adult mammalian diet (46).

The bifidobacterial glycobiome also encompasses members of GH families that are pivotal in host glycan breakdown, such as those belonging to GH33 and GH34, which represent exo-sialidases, GH29 and GH30, which represent fucosidases, and GH20, which include hexosaminidase and lacto-*N*-biosidase activities (43).

Interestingly, comparison of the bifidobacterial GH repertoire with those encoded by representatives of the main bacterial families typically residing in the human gastrointestinal tract show that the *Bifidobacteriaceae* family possesses a very broad set of genes encoding GH families GH3, GH43, GH13, and GH51, similar to (relative to genome size) the numbers found in the genomes of *Bacteroides* spp. (*Bacteroidales* family), *Clostridiales*, *Paenibacillus* spp. (*Bacillales* family), and *Streptomyces* spp. (*Actinomycetales* family) (43).

In silico evaluation of the GH repertoire of the genus *Bifidobacterium* also included the clustering of bifidobacterial (sub)species based on their predicted GH and carbohydrate degradation pathway repertoire (Fig. 4), allowing the identification of three

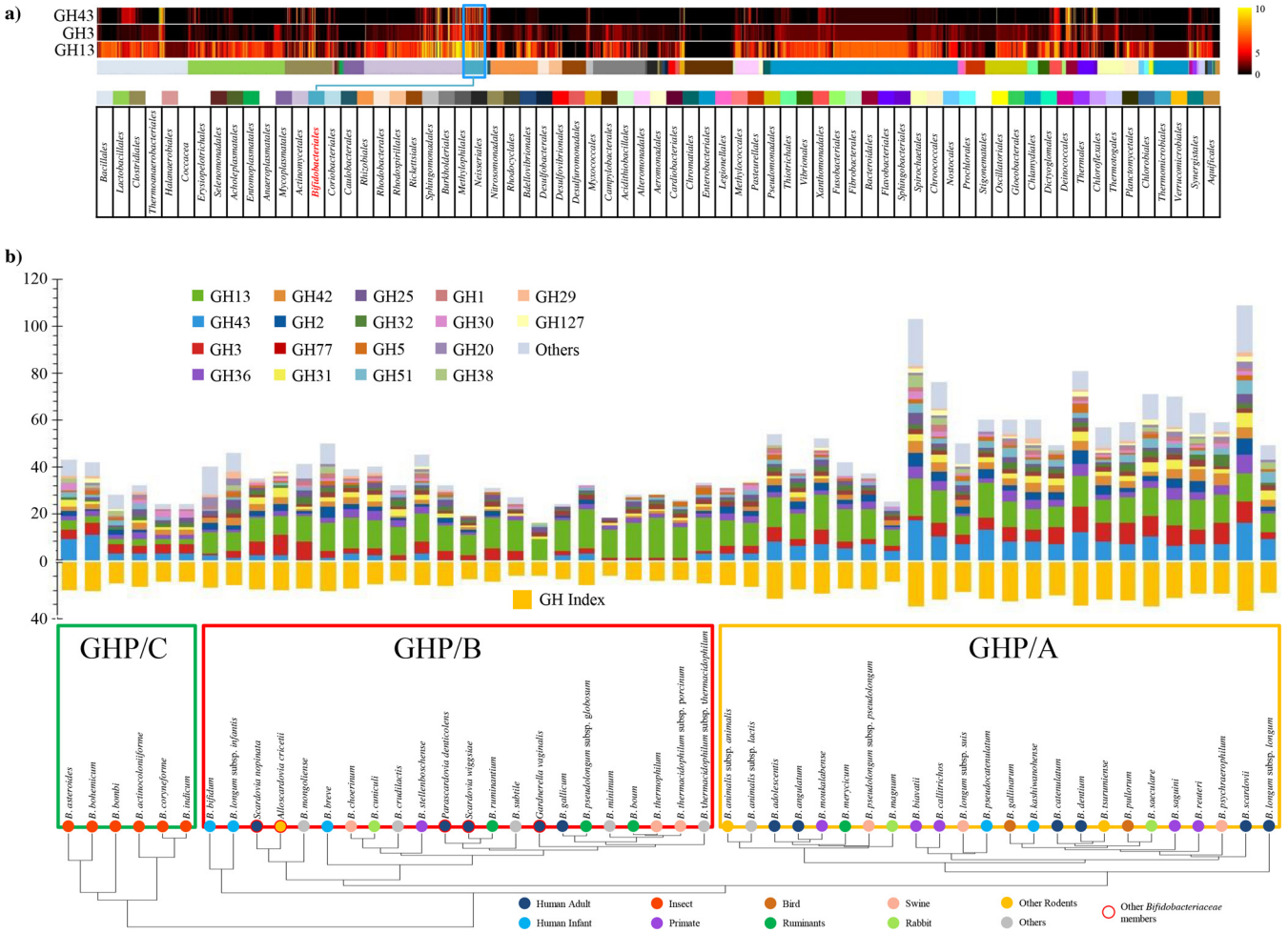

FIG 4 The glycobiome of the *Bifidobacterium* genus and some additional members of the *Bifidobacteriaceae* family. Panel a shows a comparative analysis of the bifidobacterial GH3, GH13, and GH43 repertoire against that found in other gut bacteria. The heat maps show GH prediction data from 2,721 sequenced bacterial strains belonging to bacterial orders residing in the human gut, identified by different color codes as explained in the underlying table. Data regarding *Bifidobacteriales* are highlighted in blue. In panel b, GH family profiles identified by the CAZy database were used to construct a hierarchical clustering of all tested species of the *Bifidobacterium* genus and additional members of the *Bifidobacteriaceae* family. This clustering highlights the presence of three distinct clusters named GHP/A, GHP/B, and GHP/C displaying a different repertoire of GHs. GH arsenal prediction for each analyzed *Bifidobacterium* species is represented by a bar plot, and the GH index (the number of GHs predicted in each genome normalized by genome size expressed as megabase pairs) is illustrated as an orange bar plot.

groups—designated GHP/A, GHP/B, and GHP/C (43). Notably, group GHP/A includes bifidobacterial (sub)species with a considerable array of predicted GH43 family members, representing enzymes involved in the breakdown of complex plant glycans such as xylan and arabinoxylans. This suggests adaptation of bifidobacterial (sub)species belonging to group GHP/A to hosts that enjoy a vegetarian or omnivorous diet. Bifidobacterial taxa isolated from social insects constitute the GHP/C group, as they encompass a discrete set of GH43 and GH3 family members and only a very limited number of GH13 members, which is in contrast to all other analyzed bifidobacteria (39). As described above, the GH13 family includes enzymes that are involved in the hydrolysis of poly- or oligosaccharides with α-glucosidic linkages such as starch, glycogen, and related substrates; thus, this finding can be explained by the paucity of such glycans in the (vegetarian) diet of honeybees and bumblebees. The remaining bifidobacterial (sub) species, not fitting in groups GHP/A or GHP/C, are included in group GHP/B, whose members are characterized by an underrepresentation of GH43 and GH3 enzymes (43).

As expected, part of the predicted glycobiome of bifidobacteria is extracellular, since it allows access to polysaccharides that are too large to be internalized. It is estimated that 10.9% of the total GH repertoire is located extracellularly, of which 32.9% are members of the GH13 family and annotated as pullulanases and α-amylases, and 24% are members of the GH43 family and predicted to act as β-xylosidases and α-L-arabinofuranosidases, while 12% are members of the GH51 family and classified as α-L-arabinofuranosidases (43).

Putative extracellular GHs were found in 43 (sub)species of the genus *Bifidobacterium*, with higher occurrence in the genomes of *Bifidobacterium biavatii* (predicted to encode 17 secreted GHs, including four GH43 and four GH13 members), *Bifidobacterium scardovii* (predicted to specify 11 secreted GHs, including three GH43 and three GH51 members, annotated as α-L-arabinofura-

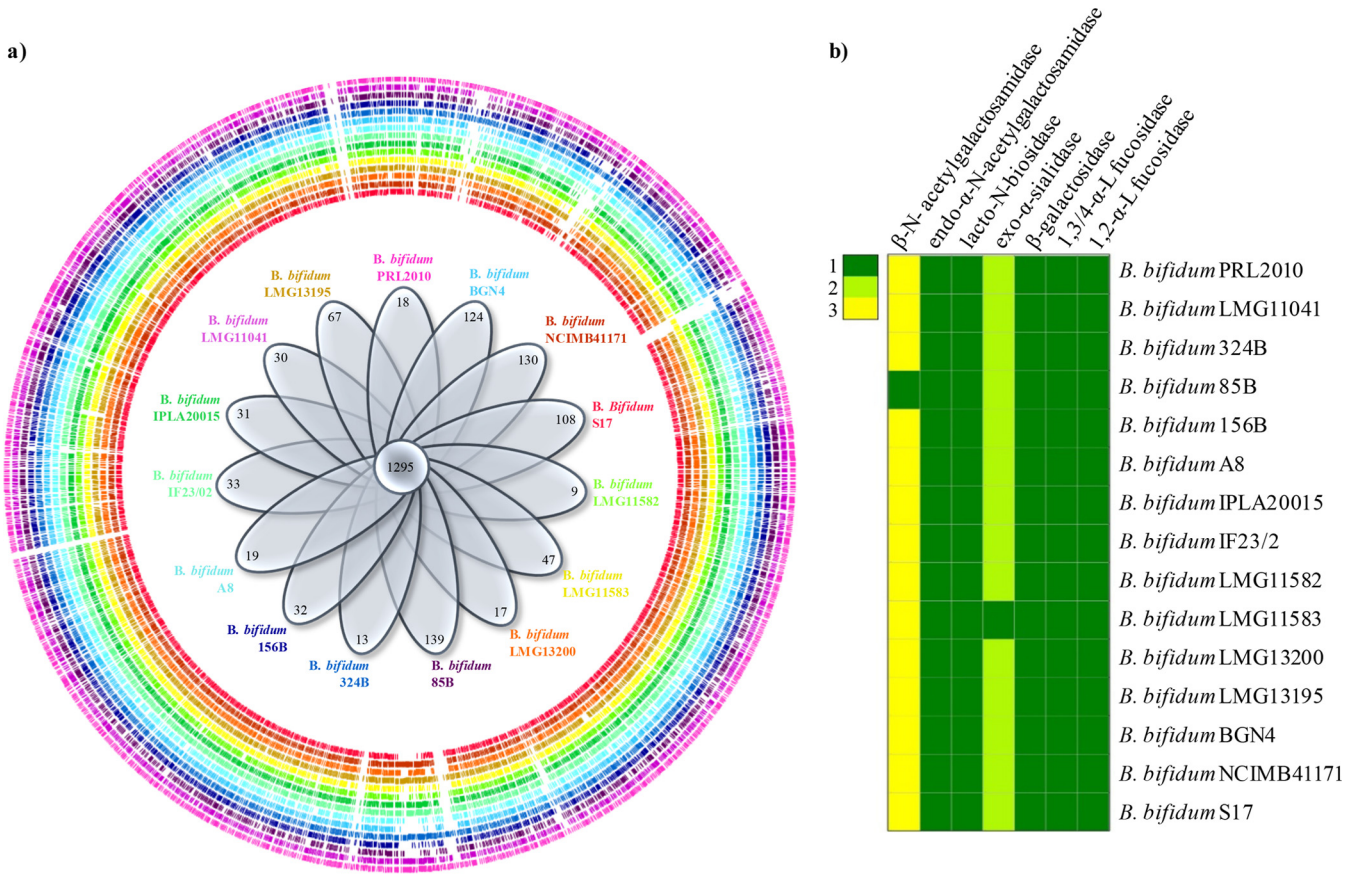

FIG 5 The pan-genome of *Bifidobacterium bifidum*. Panel a shows a genome atlas representation of all publicly available genomes of the species *B. bifidum* in which each circle represents a different strain identified by a different color. Inside the genome atlas, a Venn diagram illustrates the number of identified core and unique genes. Panel b displays a heat map that summarizes the presence and number of particular genes predicted to be involved in mucin degradation in the analyzed *B. bifidum* genomes.

nosidases), and *B. bifidum* (predicted to be endowed with 11 genes that encode secreted GHs, including two GH83 and two GH33 members, all four of which are putative sialidases) (43). The four secreted sialidases found in *B. bifidum* are clear evidence of its advanced genetic adaptation to the mammalian gut (47). In fact, sialidases are essential for the metabolism of human milk oligosaccharides (HMOs) and intestinal glycoconjugates such as mucin (see below) (20, 48, 49).

Host- or diet-derived glycans and bifidobacteria: an example of strict coevolution. Gut commensals such as bifidobacteria display a saccharolytic behavior aimed at accessing carbohydrates as their sole carbon and energy sources (43). *In silico* analyses of gut commensals have shown that the genetic arsenal dedicated to the breakdown of complex, host-indigestible carbohydrates either (in)directly derived from the host (i.e., mucin and HMO) or from the diet has had a significant impact on shaping the gut microbiome composition (50).

Mucins are host-produced glycans, secreted by intestinal goblet cells, that essentially make up the mucus layer covering the intestinal mucosa. The main monosaccharide components in mucin-derived glycoproteins are *N*-acetylglucosamine, *N*-acetylgalactosamine, and galactose, and these glycoproteins are decorated with fucose, sialic acid, and sulfate groups (51). Within the genus *Bifidobacterium*, only members of the *B. bifidum* species have been shown to efficiently degrade mucin (48, 52, 53). However, other bifidobacterial taxa, including *B. longum* subsp. *infantis*, *B. biavatii*, *B. crudilactis*, *B. kashiwanohense*, *B. stellenboschense*, and *B. mongoliense*, have recently been shown to metabolize host glycans, including mucin, though at a lower efficiency than the *B. bifidum* species (43). Further *in silico* analyses of the pan-genome of the *B. bifidum* species together with functional genome approaches revealed the existence of a gene set involved in mucin metabolism that was shown to be uniquely present in the genomes of members of this bifidobacterial species and thus constitutes the core genome sequences of the *B. bifidum* species (20, 49) (Fig. 5). Such findings represent an intriguing example of strict coevolution of a human gut commensal like *B. bifidum* to the human intestine, where the glycans produced by the host serve as carbon source for this bifidobacterial species (47). Another intriguing example of host-produced glycans that are fermented by bifidobacteria are the HMOs, which are present in human milk yet are not utilized by the (infant) host. The chromosome *B. longum* subsp. *infantis*, a typical fecal isolate from (breast-fed) infants, encompasses a gene cluster predicted to encode GHs and carbohydrate transporters necessary for the import and metabolism of HMOs (5). This 43-kb gene cluster encodes a variety of predicted or proven catabolic enzymes, such as fucosidases, sialidases, a β-hexosaminidase, and β-galactosidases, as well as extracellular

solute binding proteins and permeases that are devoted to HMO metabolism (5, 54–56). Moreover, the genome of this microorganism contains a complete urease operon predicted to be involved in the utilization of urea, representing an important nitrogen source in milk (5).

In contrast, *in silico* analyses of the genomes of two other members of the *B. longum* phylogenetic group (i.e., *B. longum* subsp. *longum* and *B. longum* subsp. *suis*) revealed a higher genomic capacity to utilize plant-derived glycans, including arabinoxylan (57, 58).

Another important sign of genetic adaptation of bifidobacteria to the human gut is the specific utilization of various complex glycans, such as resistant starches, which are derived from the diet and which escape host-mediated digestion. Starch consists of amylose and amylopectin moieties, with the former being a linear α-(1,4) glucose chain with a plant-specific degree of polymerization of 200 to 6,000, while the latter represents short linear α-(1,4)-glucose-linked chains with α-(1,6)-linked glucose side chains (59). Natural derivatives of starch are maltodextrin, maltotriose, and maltose (59). The breakdown of these complex carbohydrates is operated by gut commensals through the combined action of amylases (EC 3.2.1.1, EC 3.2.1.2, and EC 3.2.1.3) and amylopullulanases (APU [EC 3.2.1.41]). Starch is metabolized by various members of the gut microbiota, such as *Ruminococcus bromii* (60), *Bacteroides thetaiotaomicron* (61), and *Roseburia inulinivorans* (62), whose genomes encode various amylases. Even if bifidobacteria are nondominant members of the adult gut microbiota, their biological roles in the metabolism of dietary and host-derived glycans have only recently been appreciated (63). Analyses of the genome sequences of the various type strains representing each of the 47 (sub)species of the genus *Bifidobacterium* revealed the widespread occurrence of the above-mentioned starch/starch derivative-degrading enzymes (26), especially in the genomes of the adult-type *Bifidobacterium adolescentis* (31, 64) and *B. breve* (64, 65). The prediction of the glycobiome of the *B. adolescentis* species revealed, compared to most other bifidobacterial gut commensals, a much larger set of GH13 enzymes, which include amylase, pullulanase, and cyclomaltodextrinase activities, thus suggesting superior growth performance of this species on particular plant-derived carbohydrates (60). Such findings were substantiated by the analyses of fermentation profiles of members of the *B. adolescentis* taxon, which highlighted a preference for the utilization of different esose-containing sugars (e.g., galactose, mannose, and glucose), as well as plant-derived glycans that are typically present in the human diet, such as starch (31).

Among bifidobacterial species used as probiotics, *B. animalis* subsp. *lactis* deserves a special mention (25). Members of this bifidobacterial species can transit and impact resident microbial communities even if their overall effects are still not well defined (66–68). Notably, members of the *B. animalis* subsp. *lactis* taxon can only hydrolyze/metabolize very limited number of carbohydrates, perhaps underlining a rather high level of genetic adaptation to an ecological niche or perhaps due to massive genome decay as a result of its industrial exploitation, which has involved long-term cultivation of *B. animalis* subsp. *lactis* on synthetic media (25, 43).

A bifidobacterial species possessing carbohydrate breakdown capabilities toward both dietary glycans as well as host-derived glycan is represented by *Bifidobacterium breve* (65, 69–72). The reconstruction of the pan-genome of this taxon revealed a wide genetic variability for genes previously characterized as being involved in the utilization of the carbohydrates ribose, sucrose, and raffinose, as well as the plant-derived polysaccharides starch, galactan, and cellodextrin (73).

Notably, the core genome of *B. breve* encompasses genes that are predicted to encode enzymes involved in the uptake and utilization of host-derived mono/oligosaccharides, in particular those derived from mucin and HMOs (73). Examples include gene clusters predicted to be involved in the metabolism of sialic acid, lacto-*N*-biose, fucose, and *N*-linked glycans. While *B. breve* is not known to be able to grow on mucin or HMOs, host-derived mono/oligosaccharides may become available through hydrolytic activities of other (bifido)bacteria present in the gut (e.g., *B. bifidum* and *B. longum* subsp. *infantis*), allowing *B. breve* strains to utilize such liberated carbohydrates through cross-feeding activities (74–76).

Cross-feeding activities by bifidobacteria and the effect on the gut microbiota. Recently, several studies have revealed that bifidobacteria play an ecological role in shaping the gut murine microbiome toward a saccharolytic microbiota by means of cross-feeding activities (43, 74–79). Cross-feeding activities target polysaccharides that reach the gut intact, where they may undergo extracellular hydrolysis by enteric bacteria like bifidobacteria, thus generating simple glycans (i.e., monosaccharides and oligosaccharides) that may become available to other microbial gut inhabitants (80). In this context, various studies involving simple bifidobacterial communities have shown how saccharolytic bacteria may cooperate in order to obtain access to complex diet carbohydrates (e.g., starch, xylan, or arabinoxylan) (26, 43, 79) or host-derived carbohydrate (e.g., mucin and HMOs) (75, 76, 79). In such scenario, the cross-feeding activities exerted by bifidobacteria ultimately influence the gut microbiota composition as well as its functionality by enhancing the production of (certain) short-chain fatty acids (SCFA) directly or indirectly though the production of acetate, which is then converted to butyrate by a species of eubacteria (77–79).

Overall, the ability of these mutualistic/commensal activities of bifidobacteria to specifically target carbohydrates in such a sophisticated manner suggests the existence of some simplistic form of social intelligence that is aimed at regulating the dynamics of the gut microbiota relationships (43, 79).

Functional contribution of bifidobacteria to the human gut. As mentioned above, bifidobacteria are commonly isolated from the mammalian gut, with a higher prevalence during the suckling stages of life (15). However, their functional contribution to the human gut is still largely ignored. Surprisingly, in various microbiome-based studies their presence is not even detected (81) or is severely underestimated. This is likely to be linked to methodological inadequacies related to primer design and/or to sample processing (82). Nevertheless, a recent investigation of currently available metagenomic data sets did indeed identify, as expected, a variable presence of bifidobacteria, with the highest prevalence in infant-associated data sets (43). Notably, an extensive repertoire of GH-encoding genes, specifying genes encoding GH3, GH13, GH43, GH51, and GH77 members that are involved in hydrolysis of complex plant carbohydrates commonly present in the adult diet, are among the most frequently identified bifidobacterial genes in metagenomic data sets obtained from (adult) human fecal samples.

This highlights a crucial aspect of the functionality contributed by bifidobacteria in the adult gut: despite the relative paucity of these bacteria in the adult human gut, their functional contribution to the human gut microbiome is important in terms of expanding the overall glycobiome of the large intestine and may thus affect the overall gut physiology. In contrast, in the microbiomes from infant fecal samples, the high prevalence of bifidobacteria is also reflected by the abundance of bifidobacterial GH-encoding genes, such as those specifying members of the GH2, GH20, GH42, GH112, and GH129 families (43). Notably, all of these GHs are involved in the breakdown of milk-related carbohydrates such as lactose and HMOs, which represent the majority of carbohydrates present in the diet of a breast-fed infant.

CONCLUSIONS

Genome sequencing of bifidobacteria started in 2002 with the decoding of the first bifidobacterial chromosome of *B. longum* subsp. *longum* NCC2705 (18). Since then, there have been a rapidly growing number of bifidobacterial strains whose genome sequences have been decoded, providing a detailed overview of the genetic diversity among members of the *Bifidobacterium* genus, as well as generating information concerning the mechanisms by which they colonize and persist in the gastrointestinal tract. Furthermore, their relative abundance in the human gut microbiome has been substantially underestimated due to various technical issues (11, 15, 82, 83). It is expected that metagenomic studies directed to explore the composition of the microbial consortia will discover novel representatives of the *Bifidobacterium* genus. Despite such findings, many aspects of bifidobacterial biology have yet to be explored (84). This is due to the lack of molecular tools to facilitate efficient exploration of their genetic arsenal, although significant progress has been made in recent years. The continued development of tools for bifidobacteria will allow the discovery of genes as well as gene products involved in gut colonization and other host-microbe interactions, including those from which we as human beings may benefit.

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