

# Carbohydrate metabolism in Bifidobacteria

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Received: 18 October 2010 / Accepted: 15 December 2010 / Published online: 16 February 2011  
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**Abstract** Members of the genus *Bifidobacterium* can be found as components of the gastrointestinal microbiota, and are believed to play an important role in maintaining and promoting human health by eliciting a number of beneficial properties. Bifidobacteria can utilize a diverse range of dietary carbohydrates that escape degradation in the upper parts of the intestine, many of which are plant-derived oligo- and polysaccharides. The gene content of a bifidobacterial genome reflects this apparent metabolic adaptation to a complex carbohydrate-rich gastrointestinal tract environment as it encodes a large number of predicted carbohydrate-modifying enzymes. Different bifidobacterial strains may possess different carbohydrate utilizing abilities, as established by a number of studies reviewed here. Carbohydrate-degrading activities described for bifidobacteria and their relevance to the deliberate enhancement of number and/or activity of bifidobacteria in the gut are also discussed in this review.

**Keywords** Carbohydrate metabolism · Prebiotic · Probiotic · Carbohydrate · Bifidobacterial metabolism · *Bifidobacterium breve* UCC2003 · Glycosyl hydrolases

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## Introduction

For an average individual the human gastrointestinal tract (GIT) is a natural habitat for approximately  $10^{11}$ – $10^{12}$  microorganisms per gram of luminal content, collectively forming the gut microbiota with a total biomass of more than 1 kg in weight [39, 138, 156]. Metagenomic analyses allowed estimates of the total number of bacterial species that may be contained within the intestinal microbiota, ranging from approximately 500 to 1,000 distinct bacterial species [25, 34], to between 15,000 and 36,000 different species [29]. A very recent study on the minimal human gut metagenome has estimated that an individual harbours at least 160 prevalent bacterial species [99], which are also found in other individuals and which together form a complex community that colonizes the oral cavity, stomach, and small and large intestines in varying numbers [22, 58, 82]. The total number of bacterial cells is at least 10 times more than the sum of all human cells in a body [39], while the collective genome of all these bacterial cells, also termed the microbiome, consists of at least 150 times more genes than the total number of genes present in the human genome [99].

The bacterial colonization of the human GIT commences immediately after birth and is dependent on many factors, including the method of delivery (i.e. caesarian or vaginal) and feeding type of the infant (breast or formula feeding), supplementary or follow-on diet, exposure to antibiotics, hygiene conditions, and frequency and nature of illnesses, particularly gastrointestinal infections [27]. Various reports have shown that the majority of the fecal microbial population of breast-fed infants consists of bifidobacteria, with minor fractions represented by *Escherichia coli*, *Bacteroides* species and clostridia (for a review see [50]). Previous molecular analyses of the GIT

microbiota composition in healthy adults have demonstrated that most of the endogenous microorganisms are members of just two phyla, *Firmicutes* and *Bacteroides* [25, 29, 41]. Likewise, a very recent culture-independent study, which was based on sequence analysis of amplified microbial ribosomal RNA-encoding genes [16S ribosomal DNA (rDNA)], revealed that the human GIT microbiota of an adult is exclusively comprised of members that belong to five bacterial phyla: *Firmicutes* (79.4%), *Bacteroides* (16.9%), *Actinobacteria* (1%), *Proteobacteria* (0.1%) and *Verrucomicrobia* (0.1%), and that most belong to the genera *Faecalibacterium*, *Bacteroides*, *Roseburia*, *Ruminococcus*, *Eubacterium*, *Coprabacillus* and *Bifidobacterium* [130]. However, human intestinal tract chip analysis revealed that members of the *Bacteroides* phylogenetic group may be more abundant than those of the *Firmicutes* in young individuals [102], a finding which is in agreement with those published by a recent metagenomic study [99]. In addition, various studies have shown that the adult human microbiota is specific to each individual, and furthermore depends on age, diet, genetic background, physiological state, microbial interactions and environmental factors [22, 33, 102, 134, 148].

Human studies have unveiled substantial differences in the gut microbiota composition of individuals [16, 25, 135] and such differences have been linked to variations of human physiology or predisposition to disease. Research on the gut microbiota composition in humans, as supported by work in gnotobiotic mouse models, has revealed the existence of a mutualistic relationship between humans and the gut microbiota, which acts as a virtual organ to (1) influence maturation of the immune system [76, 90], (2) defend against gastrointestinal pathogens and modulate responses following epithelial cell injury [35, 42]; for a review see [104], (3) affect the host's energy balance through fermentation of non-digestible dietary fibre and anaerobic metabolism of peptides and proteins [3] as well as contributes to mammalian adiposity by regulating the metabolic network [4, 88], and (4) execute biotransformations that we are ill-equipped to perform ourselves, including processing and turn-over of xenobiotics [84, 115].

Bifidobacteria are among the prevalent groups of culturable anaerobic bacteria within the human and animal gastrointestinal tract, and among the first to colonize the human GIT, where they are thought to exert health-promoting actions, such as protective activities against pathogens via production of antimicrobial agents (e.g. bacteriocins) and/or blocking of adhesion of pathogens, and modulation of the immune response (for a review see [105]). Certain bifidobacteria are, because of these perceived health-promoting activities, commercially exploited as probiotic microorganisms. Growth and metabolic

activity of probiotic bacteria, including bifidobacteria, can be selectively stimulated by various dietary carbohydrates, which for that reason are called “prebiotics” [32, 150]. In this respect it is important to mention that over 8% of the identified genes in most studied bifidobacterial genomes are predicted to be involved in carbohydrate metabolism, which is about 30% more than what the majority of other GIT microorganisms dedicate towards utilization of such compounds [57, 59, 124, 151]. There are relatively few publications that review characterized carbohydrate hydrolases of *Bifidobacterium* sp. [141, 142, 145]. Therefore, this review will focus on the currently available knowledge on bifidobacterial carbohydrate metabolism, covering the utilization of monosaccharides, disaccharides, oligosaccharides and polysaccharides.

### Prebiotics and synbiotics as a tool to improve human health

As mentioned previously, the growth and metabolic activity of beneficial gut bacteria, such as bifidobacteria, can be selectively stimulated by non-digestible carbohydrates, termed “prebiotics”. A number of clinical studies on prebiotics and synergistic combinations of pro- and prebiotics, termed synbiotics [17], have shown that they improve general health and reduce disease risk (reviewed by [111]). However, more studies are needed to better understand the protective mechanism of prebiotics. A combination of galactooligosaccharides (GOS) and fructooligosaccharides (FOS) has been reported to reduce the incidence of atopic dermatitis and infectious episodes in infants during the first six months of life [1] and modulate the early phase of a vaccine-specific immune response in mice [154]. In addition, prebiotic short-chain FOS or FOS increases numbers of bifidobacteria and the *Eubacterium rectale-Clostridium coccoides* group in in vitro pH-controlled anaerobic faecal batch cultures [117]. Another paper recently reported that administration of FOS changes the composition of microbiota, by increasing bifidobacterial and lactobacilli counts in caecum and large intestine, and improves intestinal barrier function by upregulated expression of trefoil factor-3 and MUC2 gene [109]. Consumption of GOS has also been shown to prevent the incidence and symptoms of travelers' diarrhea [13, 24]. Recent studies on irritable bowel syndrome (IBS) treatment with trans-GOS have shown that administration of this prebiotic reduces IBS symptoms and increase the overall quality of life in patients [100, 127]. These compelling results on the beneficial effects of prebiotics also imply that characterization of carbohydrate-modifying enzymes produced by health-promoting bacteria is important as such knowledge will facilitate the

development of novel and perhaps more effective and/or selective prebiotics [141].

## Bifidobacteria

Bifidobacteria, as mentioned above, are considered to play a key role in human health. Tissier (1900) was the first to report on the isolation of a *Bifidobacterium* species (then named *Bacillus bifidus communis*) from faeces of a breast-fed infant. Bifidobacteria are Gram-positive, heterofermentative, non-motile, non-spore forming microorganisms. Due to the fact that bifidobacteria produce lactic acid as one of their main fermentation end products, they are often included in the lactic acid bacteria (LAB), even though they are phylogenetically distinct, belonging to the high G+C content (ranging from 42 to 67%) Gram-positive bacteria [6]. The family *Bifidobacteriaceae* includes the genera *Gardnerella* and *Bifidobacterium*, and belongs to the phylum and cognominal class of *Actinobacteria*, within which they form a distinct order—the *Bifidobacteriales*. The phylum *Actinobacteria* also comprises, among others, *Actinomycetaceae*, propionibacteria, corynebacteria, mycobacteria and streptomyces [103, 149]. Thirty-nine species have been assigned to the genus *Bifidobacterium*, including recent additions such as *Bifidobacterium tsurumiense* [91] isolated from hamster dental plaque, *Bifidobacterium mongoliense* isolated from airag, a traditional Mongolian fermented horse milk product [160], *Bifidobacterium crudilactis* extracted from French raw milk and raw milk cheeses [20], *Bifidobacterium psychraerophilum*, originating from pig intestine [128], and three bifidobacterial species, *Bifidobacterium bombi* [52], *Bifidobacterium actinocoloniformis* and *Bifidobacterium bohemicus* [51], isolated from the digestive tract of different bumblebee species (Table 1). Furthermore, it seems that many bifidobacteria are still to be discovered as suggested by various metagenomic studies [11, 137]. A number of such bifidobacterial strains have been commercially exploited and are usually available on the market as functional components of dairy-based probiotic drinks (Table 2).

The ‘human’ group of bifidobacterial species includes mainly those that were detected in the intestine or feces of adults or infants, and includes *Bifidobacterium pseudocatenulatum*, *Bifidobacterium catenulatum*, *Bifidobacterium adolescentis*, *Bifidobacterium longum*, *Bifidobacterium breve*, *Bifidobacterium angulatum* and *Bifidobacterium dentium* [153].

Like most intestinal bacteria, bifidobacteria are saccharolytic and are believed to play an important role in carbohydrate fermentation in the colon. Physiological data confirm that bifidobacteria can indeed ferment various

complex carbon sources such as gastric mucin, xylo-oligosaccharides, (trans)-galactooligosaccharides, soy bean oligosaccharides, malto-oligosaccharides, fructo-oligosaccharides, pectin and other plant derived-oligosaccharides, although the ability to metabolize particular carbohydrates is species- and strain-dependent [17]. In general, gut bacteria degrade polymeric carbohydrates to low molecular weight oligosaccharides, which can subsequently be degraded to monosaccharides by the use of a wide range of carbohydrate-degrading enzymes. In the case of bifidobacteria, these monosaccharides are converted to intermediates of the hexose fermentation pathway, also called fructose-6-phosphate shunt or ‘bifid’ shunt [18, 118], and ultimately converted to short chain fatty acids (SCFAs) and other organic compounds, some of which may be beneficial to the host. SCFAs, which are high in caloric content, for example, are adsorbed by the colonocytes and epathocytes, where they are metabolized and used as energy source. Besides that, SCFAs stimulate sodium and water adsorption in the colon and are known for their ability to induce enzymes that promote mucosal restitution [14].

## Bifidobacterial genomes

The genus *Bifidobacterium* sp. currently includes 39 characterized species (Table 1). The genomes of 24 different strains representing 10 bifidobacterial species have been subjected to sequence analysis, although only 10 genomes have been completely annotated (Fig. 1, Table 3). The fact that 20 bifidobacterial genomes have been sequenced during the last 3 years is a clear reflection of the significantly increased scientific interest in this particular group of bacteria, and is aimed at revealing the genetic basis for their health effects, and their colonization and persistence in the human gastrointestinal tract.

The first complete genome sequence of *B. longum* subsp. *longum* NCC2705 was published in 2002 [124]. Ten other bifidobacterial genomes including those of *B. longum* subsp. *longum* DJO10A [63], and *B. longum* subsp. *longum* JDM301 [161], *B. longum* subsp. *infantis* ATCC15697 [126], *B. adolescentis* ATCC15703, four *B. animalis* subsp. *lactis* (DSM10140, AD011, BI-04 and Bb12) [5, 30, 53], *B. bifidum* PRL2010 [136], *B. dentium* Bd1 [152] and the unpublished genome of *B. breve* UCC2003 strain have been completely sequenced and annotated (Table 3). An additional 12 bifidobacterial genome drafts are currently present in the database of the National Center for Biotechnology Information (NCBI) [131]. Furthermore, several additional genomes from *Bifidobacterium* species/strains (*B. breve* M-16 V, *B. breve* Yakult, *B. breve* UCC2003, *B. animalis* subsp. *lactis* (strains DN-173010

**Table 1** Currently recognized *Bifidobacterium* species

Bifidobacterial species	Animal origin	Reference
<i>B. actinocoloniformis</i>	Bumblebee intestine	[51, 106]
<i>B. adolescentis</i>	Adult intestine	[106]
<i>B. angulatum</i>	Human faeces, sewage	[119]
<i>B. animalis</i>		[71]
subsp. <i>animalis</i>	Chicken, rat, rabbit and calf faeces; river;	ex [120]
subsp. <i>lactis</i>	Fermented milk	ex [77]
<i>B. asteroides</i>	Hindgut of honeybee	[122]
<i>B. bifidum</i>	Adult intestine, human faeces, vagina	[93]
<i>B. bohemicus</i>	Bumblebee intestine	[51]
<i>B. bombi</i>	Bumblebee intestine	[52]
<i>B. boum</i>	Bovine rumen; piglet faeces	[121]
<i>B. breve</i>	Infant intestine, faeces, vagina	[106]
<i>B. catenulatum</i>	Child and adult intestine, vagina	[119]
<i>B. choerinum</i>	Pig faeces	[121]
<i>B. coryneforme</i>	Hindgut of honeybee	[9]
<i>B. crudilactis</i>	Raw milk and raw milk cheese	[20]
<i>B. cuniculi</i>	Rabbit faeces	[121]
<i>B. dentium</i>	Human dental caries, faeces, human vagina	[119]
<i>B. gallicum</i>	Adult intestine	(Lauer, 1990)
<i>B. gallinarum</i>	Chicken cecum	[159]
<i>B. indicum</i>	Bees; river	[122]
<i>B. longum</i>	Child and adult intestine, vagina	[72]
subsp. <i>infantis</i>	Infant intestine, vagina	ex [106]
subsp. <i>suis</i>		ex [73]
subsp. <i>longum</i>	Child and adult intestine, vagina	ex [106]
<i>B. magnum</i>	Rabbit faeces; sewage	[123]
<i>B. merycicum</i>	Rumen	[7]
<i>B. minimum</i>	Sewage	[9]
<i>B. mongoliense</i>	Fermented mare's milk product	[160]
<i>B. pseudocatenulatum</i>	Child faeces	[121]
<i>B. pseudolongum</i>		[165]
subsp. <i>globosum</i>	Pig, chicken, calf and rat faeces, rumen	ex [9]
subsp. <i>pseudolongum</i>	Pig, chicken, calf and rat faeces, rumen	ex [78]
<i>B. psychraerophilum</i>	Porcine caecum	[128]
<i>B. pullorum</i>	Chicken faeces	[133]
<i>B. ruminantium</i>	Rumen	[7]
<i>B. saeculare</i>	Rabbit faeces	[8]
<i>B. scardovii</i>	Adult faeces	[44]
<i>B. subtile</i>	Sewage	[9]
<i>B. thermophilum</i>	Pig chicken and calf faeces, bovine rumen (B. ruminale)	[78]
<i>B. thermacidophilum</i>	Anaerobic digester	[23]
<i>B. tsurumiense</i>	Hamster dental plaque	[91]

Adapted from [10]

*ex* indicates the author, who described or named a microorganism the first, by giving it a name, which a later author reclassified

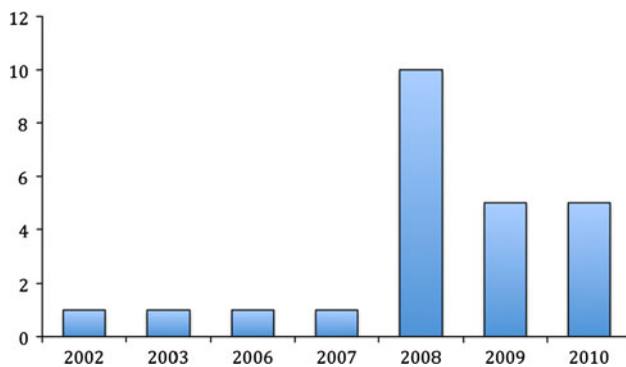
and BB-12). *B. longum* subsp. *longum* BB536 and *B. longum* subsp. *infantis* M-63) are at various stages of completion [65]. All completely and partially annotated bifidobacterial genomes range in size from 1.9 to 2.9 Mb, on which 1,604–2,588 genes have been identified and

annotated (see Table 3). The predicted functions encoded by these bifidobacterial genome sequences have provided genetic evidence that bifidobacteria are prototrophic and are very well adapted for colonization of the (human) gastrointestinal tract (Table 4).

**Table 2** Partial list of commercialized probiotic strains of *Bifidobacterium* sp

Strain	Source
<i>Bifidobacterium lactis</i> Bb-12	Chr. Hansen, Inc. (Denmark)
<i>Bifidobacterium lactis</i> FK120	Fukuchan milk (Japan)
<i>Bifidobacterium lactis</i> HN019 (DR-10)	New Zealand Dairy Board (New Zealand)
<i>Bifidobacterium lactis</i> LKM512	Fukuchan milk (Japan)
<i>Bifidobacterium (animalis) lactis</i> (same as BL Regularis)	Danone (France)
<i>Bifidobacterium longum</i> subsp. <i>longum</i> BB536a	Morinaga Milk Industry Co., Ltd. (Japan)
<i>Bifidobacterium longum</i> subsp. <i>longum</i> BB-46	Chr. Hansen, Inc. (Denmark)
<i>Bifidobacterium longum</i> subsp. <i>longum</i> SBT-2928a	Snow Brand Milk Products Co., Ltd (Japan)
<i>Bifidobacterium breve</i> strain Yakult	Yakult (Japan)
<i>Bifidobacterium</i> species 420	Danlac (Canada)

Species identification is as reported by the manufacturer, which may not reflect the current taxonomic status

**Fig. 1** Number of partially and completely sequenced *Bifidobacterium* sp. genomes available in GenBank (October 2010)

### Bifidobacterial carbohydrate metabolism

The ability of gut commensals to degrade complex carbohydrates for which their host lacks digestive capacity has been well established. These complex carbohydrates can be dietary compounds (such as resistant starches, cellulose, hemicellulose, glycogen, galactan, xylan, pullulan, pectins and gums), host-derived compounds (such as mucin, glycosphingolipids, chondroitin sulphate, hyaluronic acid and heparin) [43], or carbon sources produced by other members of the GIT microbial community [60]. The amount and nature of the non-digestible carbohydrates present in someone's diet is expected to have a direct impact on the metabolic activity, number and composition of the human GIT microbiota [156]. Gut microorganisms can utilize a diverse range of nutritional sources that escape degradation in the upper part of the GIT, as evidenced by the clear enrichment (certainly when comparing this to the human genome) for genes encoding enzymes that metabolize plant-derived oligo- and polysaccharides [156]. As expected, the genomes of gut commensals also carry more genes involved in mucin degradation than genomes of microorganisms from other environments [156].

The gene content of a bifidobacterial genome seems to reflect its adaptation to the human GIT environment, as is evident from the presence of genes that encode a variety of carbohydrate-modifying enzymes, such as glycosyl hydrolases, sugar ABC transporters, and PEP-PTS (PEP—phosphoenolpyruvate; PTS—phosphotransferase system) components, all of which are required for the metabolism of plant- and host-derived carbohydrates [5, 53, 63, 124]. As mentioned above a large proportion of the genes in a given bifidobacterial genome is predicted to be involved in sugar metabolism (Table 4), and approximately half of these are devoted to carbohydrate uptake, by means of ABC transporters, permeases and proton symporters, rather than through PEP-PTS transport [151].

The *B. longum* subsp. *longum* DJO10A genome was shown to contain 52 genes representing an estimated 10 ABC transporter systems, responsible for the uptake of various carbohydrates, as well as a putative glucose-specific PEP-PTS uptake system [67]. *In silico* analysis of the *B. longum* subsp. *longum* NCC2705 genome revealed 13 different ATP-binding cassette transporters and a single PEP-PTS system [95]. Transport of ribose, maltose, lactose, FOS,  $\alpha$ -glucosides, raffinose, mannose-containing oligosaccharides, xylose and xylosides is thought to be facilitated by ABC-type systems in this microorganism, while glucose is internalized using a glucose-specific PEP-PTS [95]. The *B. breve* UCC2003 genome is reported to contain four PEP-PTS systems, one of which is a fructose-inducible fructose/glucose-uptake PTS system (encoded by the *fru* operon) [75]. In contrast to the sequenced *B. longum* and *B. breve* strains, *B. animalis* subsp. *lactis*, whose genome is significantly smaller than that of *B. longum*, has a lower number of genes involved in the utilization of carbon sources, does not encode PEP-PTS systems and contains only two genes specifying carbohydrate-specific ATP-binding proteins typical of ABC transporters [5]. This genome reduction of industrially exploited *B. animalis* subsp. *lactis* strains may be caused by a typical genomic evolution process common to microorganisms submitted to



**Table 3** General features of completely sequenced *Bifidobacterium* genomes

Species	Genome size (bp)	GC content (%)	Genes	Proteins	Source	GeneBank no.	Reference
<i>B. adolescentis</i> ATCC15703	2,089,645	59	1,701	1,631	Human GIT	NC_008618	Unpublished
<i>B. adolescentis</i> L2-32	2,385,710	59	2,499	2,428	Infant	NZ_AAxD00000000	Unpublished
<i>B. animalis</i> subsp. <i>lactis</i> HN019	1,915,892	60	1,632	1,578	Infant	NZ_ABOT00000000	Unpublished
<i>B. animalis</i> subsp. <i>lactis</i> DSM10140	1,938,483	60	1,629	1,566	French Yogurt	NC_012815	[5]
<i>B. animalis</i> subsp. <i>lactis</i> AD011	1,933,695	60	1,604	1,528	Infant faeces	NC_011835	[53]
<i>B. animalis</i> subsp. <i>lactis</i> B1-04	1,938,709	60	1,631	1,567	Adult faeces	NC_012814	[5]
<i>B. animalis</i> subsp. <i>lactis</i> Bb12	1,942,198	61	1,624	NA	Fermented milk	CP001853	[30]
<i>B. angulatum</i> DSM20098	2,007,108	59	1,811	1,748	Human faeces	NZ_ABYS00000000	Unpublished
<i>B. bifidum</i> NCIMB41171	2,186,140	62	1,888	1,833	Adult faeces	NZ_ABQP00000000	Unpublished
<i>B. bifidum</i> PRL2010	2,214,650	59	1,848	NA	Human GIT	CP001840	[136]
<i>B. breve</i> UCC2003	2,422,668	59	1,868	1,590	Infant faeces	Unpublished	[61]
<i>B. breve</i> DSM20213	2,297,799	58	2,309	2,251	Human faeces	NZ_ACCG00000000	Unpublished
<i>B. dentium</i> ATCC27678	2,642,081	58	2,500	2,430	Dental caries	NZ_ABIX00000000	Unpublished
<i>B. dentium</i> ATCC27679	2,633,776	58	2,402	2,336	Urogenital tract	NZ_AEEQ01000000	Unpublished
<i>B. dentium</i> Bd1	~2,600,000	59	~2,270	NA	Dental caries	NC_013714	[139, 152]
<i>B. longum</i> subsp. <i>longum</i> DJO10A	2,375,792	60	2,062	1,990	Human GIT	NC_010816	[63]
<i>B. longum</i> subsp. <i>longum</i> NCC2705	2,256,640	60	1,798	1,727	Human GIT	NC_004307	[124]
<i>B. longum</i> subsp. <i>longum</i> subsp. <i>infantis</i> ATCC15697	2,832,748	59	2,588	2,416	Infant GIT	NC_011593	[126]
<i>B. longum</i> subsp. <i>longum</i> JDM301	2,477,838	59	2,035	1,958	Human faeces	NC_014169	[161]
<i>B. longum</i> subsp. <i>infantis</i> ATCC55813	2,372,858	60	2,171	2,109	Infant GIT	NZ_ACHI00000000	Unpublished
<i>B. longum</i> subsp. <i>infantis</i> CCUG52486	2,453,376	59	2,296	2,240	Infant GIT	NZ_ABQQ00000000	Unpublished
<i>B. pseudocatenulatum</i> DSM20438	2,304,808	56	2,220	2,151	Human faeces	NZ_ABXX00000000	Unpublished
<i>B. catenulatum</i> DSM16992	2,058,429	56	2,011	1,950	Human faeces	NZ_ABXY00000000	Unpublished
<i>B. gallicum</i> DSM20093	2,016,380	57	2,045	1,983	Human faeces	NZ_ABXB00000000	Unpublished

extended cultivation under artificial conditions such as those of industrial batch fermentation [63]. The human intestine is one of such environments, where large amounts of energy sources and metabolic intermediates produced by other members of microbiota are available for utilization.

As mentioned above, bifidobacteria degrade hexose sugars through a particular metabolic pathway, termed the “bifid shunt”, where the fructose-6-phosphoketolase enzyme (EC 4.1.2.2) plays a key role (Fig. 2) [18]. This enzyme is considered to be a taxonomic marker for the family of *Bifidobacteriaceae* [28]. Additional enzymes are needed to channel various diet- and host-derived carbon sources into this so-called “bifid shunt” (Fig. 2), which allows bifidobacteria to produce more energy in the form of ATP from carbohydrates than the fermentative pathways operating in, for example, lactic acid bacteria, because the bifidobacterial pathway yields 2.5 ATP molecules from 1 mol of fermented glucose, as well as 1.5 mol of acetate and 1 mol of lactate [94]. In contrast, the homofermentative group of lactic acid bacteria (LAB) produces 2 mol of ATP and 2 mol of lactic acid from 1 mol of glucose,

whereas heterofermentative LAB produce 1 mol each of lactic acid, ethanol and ATP per 1 mol of fermented glucose [116]. The ratio of lactate to acetate formed by bifidobacteria may vary depending on the carbon source utilized and also on the species examined [94].

Carbohydrate metabolic abilities may vary considerably between bifidobacterial strains [70], as was confirmed by recent studies (Table 5). However, many of the characterized strains can utilize ribose, galactose, fructose, glucose, sucrose, maltose, melibiose and raffinose, but generally cannot ferment L-arabinose, rhamnose, N-acetylglucosamine, sorbitol, melezitose, trehalose, glycerol, xylitol and inulin (Table 5). Only a minority of the sugars utilized by bifidobacteria are believed to be internalized via a PEP-PTS [19, 75], while uptake of most of the remaining, more complex sugars is possibly facilitated through the use of specific ABC transporters [124]. Following internalization such carbohydrates can then be hydrolysed, phosphorylated, deacetylated and/or transglycosylated by dedicated intracellular enzymes (Table 6) [142, 151].

**Table 4** Protein distribution by COG functional categories of genomes from bifidobacteria and other groups of human intestinal bacteria (October 2010)

COG description	% in <i>Bifidobacterium</i> <sup>a</sup>	% in <i>Bacteroides</i> <sup>a</sup>	% in <i>Lactobacillus</i> <sup>a</sup>	% in Actinobacteria <sup>a</sup>	% in Bacteria <sup>a</sup>
Translation	6.3993	3.4093	6.9565	3.371	4.2968
RNA processing and modification	0.0758	0	0	0.0229	0.0148
Transcription	6.5036	4.6561	7.343	7.1218	5.9643
Replication, recombination and repair	6.5131	4.4224	6.1353	4.3295	4.8261
Chromatin structure and dynamics	0	0.0195	0	0.0104	0.0269
Cell cycle control, mitosis and meiosis	1.204	0.565	1.256	0.6397	0.765
Nuclear structure	0	0	0	0	0
Defense mechanisms	2.4649	2.143	2.2222	1.1668	1.259
Signal transduction mechanisms	3.2328	3.4288	2.7536	3.2459	3.9952
Cell wall/membrane biogenesis	3.6405	6.5848	4.3961	2.885	4.3931
Cell motility	0.0948	0.0584	0.2899	0.1722	1.4575
Cytoskeleton	0.0095	0	0	0.0145	0.0116
Extracellular structures	0	0	0	0	0.0154
Intracellular trafficking and secretion	0.7774	1.4027	1.0628	0.6363	1.7757
Posttranslational modification, protein turnover, chaperones	2.5218	1.9287	2.5121	2.2143	2.929
Energy production and conversion	2.3606	3.1366	3.6232	4.8755	4.8217
Carbohydrate transport and metabolism	8.7505	5.1822	8.8889	5.1296	4.9545
Amino acid transport and metabolism	9.5942	4.5782	7.4396	6.9266	7.2726
Nucleotide transport and metabolism	3.1191	1.5196	3.6232	1.6305	1.7769
Coenzyme transport and metabolism	2.3132	2.5716	1.401	2.8294	2.97
Lipid transport and metabolism	1.8771	1.5391	1.7391	5.0221	2.8606
Inorganic ion transport and metabolism	4.3989	4.8704	4.2029	4.3659	4.5898
Secondary metabolites biosynthesis, transport and catabolism	0.493	0.8572	0.5797	4.5415	2.2675
General function prediction only	10.0588	8.5525	10.5797	11.4499	10.4609
Function unknown	4.8066	4.0327	6.9082	4.8	6.14
Not in COGs <sup>b</sup>	18.7903	34.5412	16.087	22.5988	20.1551

<sup>a</sup> These data were retrieved from NCBI (<http://www.ncbi.nlm.nih.gov/>)

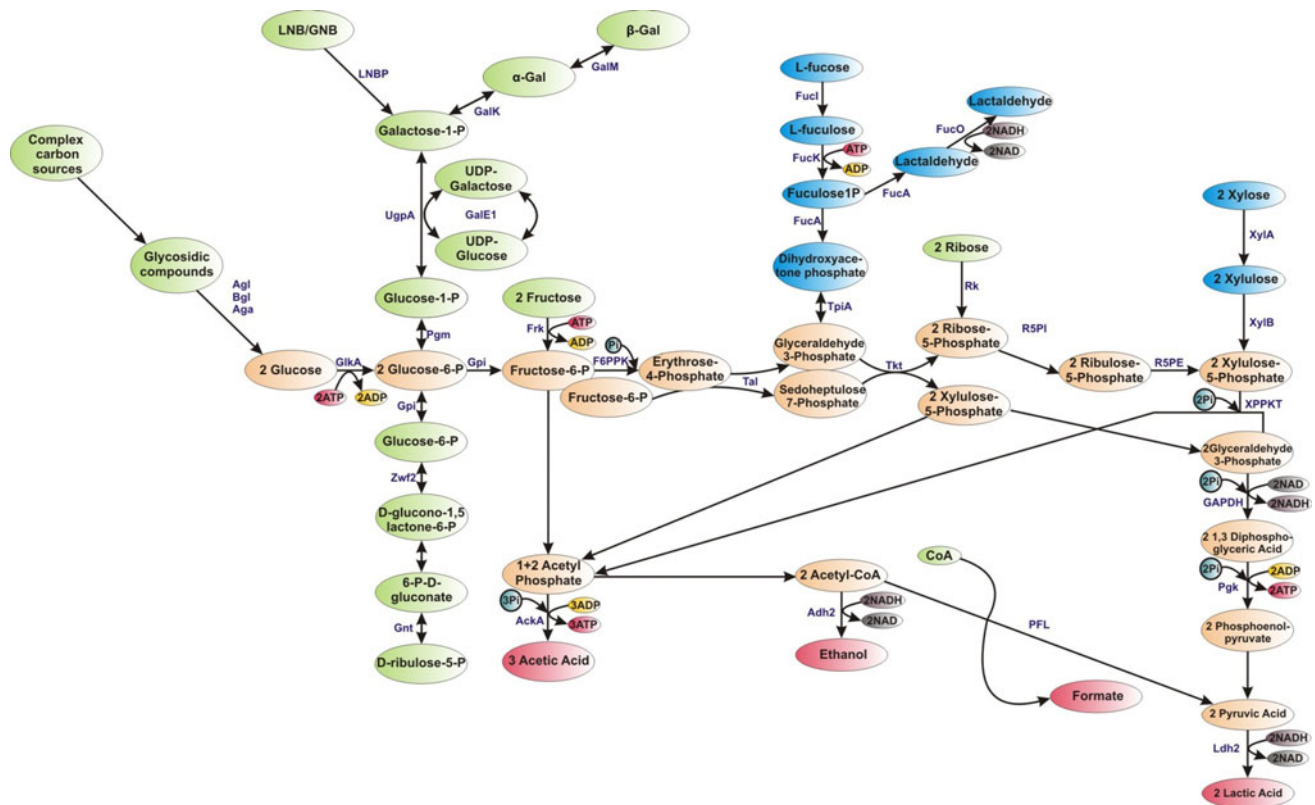
<sup>b</sup> Not in COGs category includes mainly hypothetical protein

Analysis of recently sequenced bifidobacterial genomes disclosed intriguing insights into the relationship between these bacteria and their human host. For example, *B. longum* subsp. *infantis* ATCC15697 isolated from infant feces was demonstrated to possess a 43 kb gene cluster responsible for transport and utilization of non-digestible human milk oligosaccharides (HMOs), which may explain why this micro organism predominates in the gut of breast-fed infants [126]. *B. animalis* subsp. *lactis* strains and *B. breve* UCC2003, on the other hand, do not appear to encode the genes that allow HMO degradation, but instead are able to degrade complex plant-derived oligosaccharides, a clear indication of niche-specific adaptation [5, 87, 98, 113]. In addition, analysis of the recently sequenced and annotated genome of *B. bifidum* PRL2010 strain, isolated from an infant stool, revealed a set of chromosomal loci that targets host-derived glycans [136]. These O-linked glycans are

known to be present in highly glycosylated proteins, also called mucins, produced by intestinal epithelial tissue.

### Carbohydrate-modifying enzymes in *Bifidobacterium* sp.

Enzymes are single- or multi-chain proteins that catalyze specific chemical reactions [162], and are classified into six different classes with each enzyme assigned a unique four-figure code (EC number; IUB 1984), based on the nature of the catalyzed chemical reaction. Oligo- and polysaccharides can be modified by a range of different enzymes like hexosyl- and phosphotransferases, hydrolases and isomerases. Glycosyl hydrolases (also called glycoside hydrolases) appear to be the most critical group of enzymes for bifidobacteria, which allows them to adapt to and exist in



**Fig. 2** Schematic representation of carbohydrate degradation through “bifid shunt” in bifidobacteria. Abbreviations: AckA, acetate kinase; Adh2, aldehyde-alcohol dehydrogenase 2; Aga,  $\alpha$ -galactosidase; Agl,  $\alpha$ -glucosidase; Bgl,  $\beta$ -glucosidase; GalE1, UDP-glucose 4-epimerase; GalK, galactokinase; GalM, galactose mutarotase; GAPDH, glyceraldehyde-3-phosphate dehydrogenase C; GlkA, glucokinase; Gnt, 6-phosphogluconate dehydrogenase; Gpi, glucose 6-phosphate isomerase; Frk, fruktokinase; F6PPK, fructose-6-phosphoketolase; FucI, L-fucose isomerase; FucK, L-fuculose kinase; FucA, L-fuculose-1P

aldolase;FucO, lactaldehyde reductase; Ldh2, lactate dehydrogenase; LNBp, lacto-*N*-biose phosphorylase;Pgg, phosphoglyceric kinase; Pgm, phosphoglucomutase; Pfl, formate acetyltransferase; Rk, ribokinase; R5PI, ribose-5-phosphate isomerase; R5PE, ribulose-5-phosphate epimerase; Tal, transaldolase; Tkt, transketolase; TpiA, triosephosphate isomerase;UgpA, UTP-glucose-1-phosphate uridylyltransferase; XPPKT, xylulose-5-phosphate/fructose-6-phosphate phosphoketolase; XylA, xylose isomerase;XylB, xylulose kinase; Zwf2, glucose-6-phosphate 1-dehydrogenase; Pi, phosphate

the host environment through hydrolysis of complex dietary and host-produced carbohydrates.

Glycoside hydrolases (EC 3.2.1.x) are a widespread group of enzymes, which hydrolyze the glycosidic bond between two or more carbohydrates, or between a carbohydrate and a non-carbohydrate moiety in the presence of water. The IUB-MB enzyme nomenclature of glycoside hydrolases (GH) is based on substrate specificity and occasionally on their molecular mechanism, and does not take the structural features of these enzymes into account. A classification of glycoside hydrolases in families based on amino acid sequence similarities has been proposed a couple of years ago (<http://afmb.cnrs-mrs.fr/CAZY/index.html>). Usually, hydrolysis of the glycosidic bond is performed by two catalytic residues of the enzyme: a general acid (proton donor) and a nucleophile/base. Depending on the spatial position of these catalytic residues, hydrolysis occurs via overall retention or inversion of the anomeric configuration [15]. Retaining glycoside hydrolases use a

double displacement mechanism to catalyze hydrolysis with retention of configuration at the anomeric center, which can also yield elongated oligosaccharides with new types of linkages, a reaction which is termed transglycosylation [163]; Fig. 3). When a high concentration of sugar is used in the reaction, retaining enzymes can use the carbohydrate molecule as an incoming nucleophile instead of water, which results in the exchange of the sugar residues and can lead to the formation of new oligosaccharides with a higher degree of polymerization [142]. Inverting glycoside hydrolases always use water as the acceptor molecule (single displacement mechanism) and are therefore incapable of performing transglycosylation [142].

Among the currently characterized bifidobacterial glycoside hydrolases are  $\alpha$ -galactosidases (GH family 36),  $\beta$ -galactosidases (GH family 2 or 42), or enzymes active towards gluco-oligosaccharides, such as  $\alpha$ -glucosidases and sucrose phosphorylases (both enzymes belong to GH family 13) (Table 6). As mentioned above, due to the



**Table 5** The fermentative characteristics of several species from the genus *Bifidobacterium*

Strain name	L-Arabinose	D-Ribose	D-Xylose	D-Galactose	D-Fructose	D-Mannose	D-Glucose	L-Rhamnose	N-acetylglucosamine	Sorbitol	Salicin	D-Lactose	Cellobiose
<i>B. breve</i> UCC2003*	-	+	-	+	+	±	+	-	±	-	±	+	+
<i>B. breve</i> JCM7017*	-	+	NA	+	+	NA	+	NA	NA	NA	NA	NA	NA
<i>B. breve</i> JCM7019*	-	+	NA	±	+	NA	+	NA	NA	NA	NA	NA	NA
<i>B. breve</i> NCFB2258*	-	+	NA	+	+	NA	+	NA	NA	NA	NA	NA	NA
<i>B. breve</i> NCIMB8815*	-	+	NA	+	+	NA	+	NA	NA	NA	NA	NA	NA
<i>B. breve</i> NCTC11815*	-	±	NA	+	+	NA	+	NA	NA	NA	NA	NA	NA
<i>B. breve</i> NCFB2257*	-	+	NA	+	+	NA	+	NA	NA	NA	NA	NA	NA
<i>B. longum</i> subs. <i>longum</i> NCC2705	+	+	+	+	+	-	+	-	-	-	-	+	NA
<i>B. tsurumense</i> OMB115	+	+	+	+	+	+	+	-	-	-	+	+	+
<i>B. boum</i> JCM1211	-	-	-	+	+	-	+	-	-	-	-	-	-
<i>B. thermacidophilum</i> JCM11165	+	-	-	+	+	±	+	-	-	-	-	-	-
<i>B. indicum</i> JCM1302	-	+	-	+	+	-	+	-	-	-	+	-	+
<i>B. dentium</i> JCM1195	+	+	+	+	+	+	+	-	-	-	+	+	+
<i>B. psychraerophilum</i> LMG21775	+	NA	+	NA	NA	-	NA	NA	NA	NA	+	NA	+
<i>B. psychraerophilum</i> YIT11814	+	+	+	+	+	-	+	-	-	-	+	+	+
<i>B. minimum</i> JCM5821	-	NA	-	NA	NA	-	NA	NA	NA	NA	-	NA	-
<i>B. minimum</i> YIT4097	-	-	-	-	+	-	+	-	-	-	-	-	-
<i>B. asteroides</i> JCM8230	+	NA	±	NA	NA	±	NA	NA	NA	NA	+	NA	+
<i>B. coryneforme</i> JCM5819	+	NA	+	NA	NA	-	NA	NA	NA	NA	+	NA	+
<i>B. indicum</i> JCM1302	+	NA	-	NA	NA	±	NA	NA	NA	NA	+	NA	+
<i>B. crudilactis</i> LMG 23609	-	+	-	+	+	-	+	-	-	-	NA	+	NA
<i>B. mongoliense</i> YIT10443	+	+	-	+	-	-	+	-	-	-	+	+	+
<i>B. mongoliense</i> YIT10738	+	-	-	+	±	±	+	-	-	-	-	+	-
Strain name	Melibiose	Melezitose	Turanose	Sucrose	Maltose	Trehalose	Raffinose	Mannitol	Glycerol	Xylitol	Starch	Glycogen	Inulin
<i>B. breve</i> UCC2003*	+	+	+	+	+	-	+	NA	-	-	+	+	-
<i>B. breve</i> JCM7017*	NA	NA	NA	+	NA	NA	NA	NA	NA	NA	NA	NA	NA
<i>B. breve</i> JCM7019*	NA	NA	NA	-	NA	NA	NA	NA	NA	NA	NA	NA	NA
<i>B. breve</i> NCFB2258*	NA	NA	NA	+	NA	NA	NA	NA	NA	NA	NA	NA	NA
<i>B. breve</i> NCIMB8815*	NA	NA	NA	-	NA	NA	NA	NA	NA	NA	NA	NA	NA
<i>B. breve</i> NCTC11815*	NA	NA	NA	+	NA	NA	NA	NA	NA	NA	NA	NA	NA
<i>B. breve</i> NCFB2257*	NA	NA	NA	±	NA	NA	NA	NA	NA	NA	NA	NA	NA
<i>B. longum</i> subs. <i>longum</i> NCC2705	+	NA	+	+	+	-	+	-	-	-	-	NA	NA
<i>B. tsurumense</i> OMB115	+	-	+	+	+	+	+	+	-	-	+	+	-
<i>B. boum</i> JCM1211	+	-	+	+	+	-	+	-	-	-	+	+	-
<i>B. thermacidophilum</i> JCM11165	+	-	+	+	+	-	+	-	-	-	+	+	-
<i>B. indicum</i> JCM1302	+	-	+	+	+	-	+	-	-	-	+	+	-
<i>B. dentium</i> JCM1195	+	-	+	+	+	-	+	±	-	-	-	-	-
<i>B. psychraerophilum</i> LMG21775	NA	+	NA	+	-	NA	+	NA	NA	NA	NA	NA	NA

Table 5 continued

Strain name	Melibiose	Melezitose	Turanose	Sucrose	Maltose	Trehalose	Raffinose	Mannitol	Glycerol	Xylitol	Starch	Glycogen	Inulin
<i>B. psychraerophilum</i> YIT11814	+	+	+	+	+	-	+	-	-	-	-	-	-
<i>B. minimum</i> JCM5821	NA	-	NA	NA	+	NA	-	NA	NA	NA	NA	NA	NA
<i>B. minimum</i> YIT4097	-	-	+	+	+	-	-	-	-	-	+	+	-
<i>B. asteroides</i> JCM8230	NA	-	NA	NA	±	NA	+	NA	NA	NA	NA	NA	NA
<i>B. coryneforme</i> JCM5819	NA	-	NA	NA	+	NA	+	NA	NA	NA	NA	NA	NA
<i>B. indicum</i> JCM1302	NA	-	NA	NA	±	NA	+	NA	NA	NA	NA	NA	NA
<i>B. crudilactis</i> LMG 23609	+	-	+	+	+	-	+	-	-	-	-	-	-
<i>B. mongoliense</i> YIT10443	+	-	+	+	+	-	+	-	-	-	+	+	-
<i>B. mongoliense</i> YIT10738	+	-	+	+	+	-	+	-	-	-	±	+	-

Symbols: +, positive; -, negative; ± weak growth; NA, no information available\*; , unpublished data from K. Pokusaeva & D. Van Sinderen. (Adapted from [125])

typical mechanism of action the retaining enzymes can also be used for the formation of glycosidic linkages instead of hydrolysis. Oligosaccharides synthesized in this manner may be used as a prebiotic if they are selectively metabolized by probiotic (bifido)bacteria present in the colon.

$\beta$ -galactosidases represent the most common (Table 6) as well as the best studied group of bifidobacterial glycosyl hydrolases with transglycosyl activity that can be used for the synthesis of prebiotic substances from lactose [101]. This enzymatic activity is essential for bifidobacteria as it allows them to grow on milk or milk-derived substrates, including lactose, lactose-derived (trans)galactooligosaccharides that contain  $\beta$ -galactosidic linkages [142]. The  $\beta$ -galactosidase hydrolytic and transglycosyl activities have been demonstrated and described for various bifidobacterial species, including those of *B. angulatum* [101], *B. adolescentis* [40], *B. bifidum* [37, 49, 80], *B. longum* [45, 110], *B. longum* subsp. *infantis* [46, 80] and *B. pseudolongum* [101]. *B. bifidum* NCIMB41171 encodes four different  $\beta$ -galactosidases with apparently different biochemical characteristics and with hydrolytic as well as transglycosyl activities [36, 37]. The ability of these enzymes to hydrolyze different substrates (e.g.  $\beta$ -D-(1  $\rightarrow$  6) galactobiose,  $\beta$ -D-(1  $\rightarrow$  4) galactobiose,  $\beta$ -D-(1  $\rightarrow$  4) galactosyl-lactose and *N*-acetyllactosamine) contributes in a different way to the physiology of this microorganism [36] and provides advantageous properties for a better adaptation in a carbohydrate-rich GIT environment. Oral administration of galactooligosaccharides, produced by different  $\beta$ -glucosidases isolated from *B. bifidum* NCIMB41171 [140], significantly increases bifidobacterial numbers in the stool of healthy individuals, suggesting that these carbohydrates have bifidogenic properties and can successfully be used as prebiotic [21]. In addition, the characterized  $\beta$ -galactosidase from *B. bifidum* NCIMB 41171 was shown to degrade human milk oligosaccharides lacto-*N*-tetraose (LNT) and lacto-*N*-neotetraose more rapidly than lactose [79].

Interestingly, galacto-*N*-biose (GNB)/lacto-*N*-biose (LNB) I phosphorylase (GLNBP) was shown to be responsible for LNB degradation in all *B. longum* subsp. *longum*, *B. longum* subsp. *infantis*, *B. breve*, and *B. bifidum*, strains, which are the predominant species in infant intestines [55, 164]. Therefore, LNB is presumed to be a natural prebiotic for bifidobacteria [56]. The use of recombinant GLNBP from *B. bifidum* for large scale production of GNB, LNB and their derivatives, has recently been achieved [155, 166] and it will be very interesting to determine their prebiotic potential. In this content, it is worthy mentioning that studying bifidobacterial carbohydrate-degrading enzymes is not only important in order to identify and understand preferable bifidobacterial substrates, but also from a perspective of discovering novel selective (bifidogenic) prebiotics.

**Table 6** Putative carbohydrate-modifying enzymes identified in the genome sequences of *Bifidobacterium*

EC number	Enzyme name	<i>B. adolescentis</i> ATCC15703	<i>B. adolescentis</i> L2-32	<i>B. angulatum</i> DSM20098	<i>B. animalis</i> subsp. <i>lactis</i> AD1011	<i>B. animalis</i> subsp. <i>lactis</i> 04	<i>B. animalis</i> subsp. <i>lactis</i> BI-HN019	<i>B. animalis</i> subsp. <i>lactis</i> HN019	<i>B. bifidum</i> NCUMB41171	<i>B. breve</i> DSM20213	<i>B. catenulatum</i> DSM16992
<i>Hexosyltransferases</i>											
EC:2.4.1.1	Phosphorylase	1	1	1	1	NA	1	1	1	-	1
EC:2.4.1.7	Sucrose phosphorylase.	1	-	1	1	1	1	1	-	1	1
EC:2.4.1.18	1,4-alpha-glucan branching enzyme	1	1	1	1	NA	-	-	1	1	1
EC:2.4.1.25	4-alpha-glucanotransferase	2	2	2	2	NA	2	2	1	2	2
EC:2.4.1.230	Kojibiose phosphorylase	-	-	-	-	NA	-	-	-	-	-
<i>Phosphotransferases</i>											
EC:2.7.1.12	Gluconokinase.	1	1	-	-	NA	-	-	-	-	1
EC:2.7.1.15	Ribokinase.	1	1	2	1	NA	1	1	-	2	1
EC:2.7.1.17	Xylulokinase.	1	1	1	1	NA	1	1	-	-	1
EC:2.7.1.31	Glycerate kinase.	1	1	1	-	NA	-	-	1	1	1
EC:2.7.1.4	Fructokinase.	1	1	1	1	NA	1	1	1	1	1
EC:2.7.1.6	Galactokinase.	1	2	1	1	NA	1	1	1	6	1
EC:2.7.6.1	Ribose-phosphate diphosphokinase.	2	2	2	2	NA	2	2	2	2	2
<i>Glycosyl hydrolases</i>											
EC:3.2.1.1	$\alpha$ -amylase.	1	1	-	1	-	1	1	-	1	-
EC:3.2.1.10	Oligo-1,6-glucosidase.	1	2	1	1	1	1	1	-	2	-
EC:3.2.1.14	Chitinase	-	-	-	-	-	-	-	-	-	-
EC:3.2.1.18	Exo-alpha-sialidase	-	-	-	-	NA	-	-	-	1	-
EC:3.2.1.20	Alpha-glucosidase.	3	3	1	3	1	2	2	1	2	1
EC:3.2.1.21	Beta-glucosidase.	5	4	2	3	2	3	3	-	4	4
EC:3.2.1.22	Alpha-galactosidase.	2	4	2	2	2	2	2	1	1	3
EC:3.2.1.23	Beta-galactosidase.	5	5	4	4	1	4	4	3	4	3
EC:3.2.1.24	Alpha-mannosidase	1	-	-	-	-	-	-	-	2	1
EC:3.2.1.25	Beta-mannosidase	-	-	-	-	1	-	-	-	-	-
EC:3.2.1.26	Beta-fructofuranosidase	1	1	1	1	1	1	1	-	1	1
EC:3.2.1.31	Beta-glucuronidase	-	-	-	-	-	-	-	-	-	-
EC:3.2.1.35	Hyaluronoglucosaminidase	-	-	-	-	-	-	-	1	-	-
EC:3.2.1.37	Xylan-1,4-beta-xylosidase	2	1	1	1	2	1	1	-	-	3
EC:3.2.1.4	Cellulase	1	1	1	-	-	-	-	-	-	1
EC:3.2.1.41	Pullulanase	1	1	1	-	NA	-	-	-	1	-
EC:3.2.1.45	Glucosylceramidase	1	1	-	2	2	2	2	-	-	1
EC:3.2.1.51	Alpha-L-fucosidase	-	-	-	-	NA	-	-	-	-	-

Table 6 continued

EC number	Enzyme name	<i>B. adolescentis</i> ATCC15703	<i>B. adolescentis</i> L2-32	<i>B. angulatum</i> DSM20098	<i>B. animalis</i> subsp. <i>lactis</i> AD1011	<i>B. animalis</i> subsp. <i>lactis</i> 04	<i>B. animalis</i> BI-subsp. <i>lactis</i> HN019	<i>B. animalis</i> NCUMB41171	<i>B. breve</i> DSM20213	<i>B. catenulatum</i> DSM16992
EC:3.2.1.52	Beta-N-acetylhexosaminidase	1	1	1	1	NA	1	3	1	1
EC:3.2.1.54	Cyclomaltodextrinase	-	-	-	-	NA	-	-	1	-
EC:3.2.1.55	Alpha-N-arabinofuranosidase	2	-	1	1	1	1	-	-	3
EC:3.2.1.78	Mannan endo-1,4-beta-mannosidase	-	-	-	1	NA	1	-	-	-
EC:3.2.1.86	6-phospho-beta-glucosidase	-	-	-	-	NA	-	1	-	-
EC:3.2.1.89	Arabinogalactan endo-1,4-beta-galactosidase	-	-	-	-	-	-	-	-	-
EC:3.2.1.93	Trehalose-6-phosphate hydrolase	3	-	-	-	1	-	-	-	-
EC:3.2.1.96	Mannosyl-glycoprotein endo-beta-N-acetylglucosaminidase	-	-	-	-	NA	-	-	-	-
<i>Isomerases</i>										
EC:5.3.1.1	Triosephosphate isomerase	1	1	1	1	NA	1	1	1	1
EC:5.3.1.4	L-arabinose isomerase	1	1	1	1	NA	1	-	-	1
EC:5.3.1.5	Xylose isomerase	1	1	1	1	NA	1	-	-	1
EC:5.3.1.6	Ribose-5-phosphate isomerase	1	1	1	1	NA	1	1	1	1
EC:5.3.1.9	Glucose-6-phosphate isomerase	1	1	1	1	NA	1	1	1	1
EC number	Enzyme name	<i>B. dentium</i> ATCC27678	<i>B. gallicum</i> DSM20093	<i>B. longum</i> subsp. <i>longum</i> ATCC15697	<i>B. longum</i> subsp. <i>Infantis</i> ATCC55813	<i>B. longum</i> subsp. <i>Infantis</i> CCUG52486	<i>B. longum</i> subsp. <i>longum</i> DJO10A	<i>B. longum</i> subsp. <i>longum</i> NCC2705	<i>B. longum</i> subsp. <i>longum</i> DSM20438	<i>B. pseudocatenulatum</i> DSM20438
<i>Hexosyltransferases</i>										
EC:2.4.1.1	Phosphorylase	1	1	1	1	1	1	1	1	1
EC:2.4.1.7	Sucrose phosphorylase	1	-	1	1	1	1	1	1	1
EC:2.4.1.18	1,4-alpha-glucan branching enzyme	1	1	1	1	1	1	1	1	1
EC:2.4.1.25	4-alpha-glucanotransferase	2	2	2	2	2	2	2	2	2
EC:2.4.1.230	Kojibiose phosphorylase	-	-	-	-	-	-	-	-	-
<i>Phosphotransferases</i>										
EC:2.7.1.12	Gluconokinase	1	-	-	1	1	1	1	1	-
EC:2.7.1.15	Ribokinase	1	1	2	4	2	2	2	2	1

Table 6 continued

EC number	Enzyme name	<i>B. dentium</i> ATCC27678	<i>B. gallicum</i> DSM20093	<i>B. longum</i> subsp. <i>longum</i> ATCC15697	<i>B. longum</i> subsp. <i>Infantis</i> ATCC55813	<i>B. longum</i> subsp. <i>Infantis</i> CCUG52486	<i>B. longum</i> subsp. <i>longum</i> DJO10A	<i>B. longum</i> subsp. <i>longum</i> NCC2705	<i>B. pseudocatenulatum</i> DSM20438
EC:2.7.1.17	Xylulokinase.	1	1	1	2	2	2	2	2
EC:2.7.1.31	Glycerate kinase.	1	-	1	1	1	1	1	1
EC:2.7.1.4	Fructokinase.	1	1	1	2	1	1	1	1
EC:2.7.1.6	Galactokinase.	1	1	1	1	1	1	1	1
EC:2.7.6.1	Ribose-phosphate diphosphokinase.	2	2	2	2	2	2	2	2
<i>Glycosyl hydrolases</i>									
EC:3.2.1.1	$\alpha$ -amylase.	-	-	1	-	-	-	-	-
EC:3.2.1.10	Oligo-1,6-glucosidase.	1	1	1	-	2	1	1	2
EC:3.2.1.14	Chitinase	-	-	-	-	-	-	1	1
EC:3.2.1.18	Exo-alpha-sialidase	-	-	2	-	-	-	-	-
EC:3.2.1.20	Alpha-glucosidase.	4	1	1	2	1	2	1	1
EC:3.2.1.21	Beta-glucosidase.	12	1	2	3	2	2	1	4
EC:3.2.1.22	Alpha-galactosidase.	5	-	1	1	1	1	1	2
EC:3.2.1.23	Beta-galactosidase.	4	1	4	4	3	3	3	5
EC:3.2.1.24	Alpha-mannosidase	1	-	2	2	-	3	3	-
EC:3.2.1.25	Beta-mannosidase	-	-	-	-	-	-	-	-
EC:3.2.1.26	Beta-fructofuranosidase	2	1	1	1	1	1	1	1
EC:3.2.1.31	Beta-glucuronidase	1	-	-	-	1	1	-	-
EC:3.2.1.35	Hyaluronoglucosaminidase	-	-	-	-	-	-	-	-
EC:3.2.1.37	Xylan-1,4-beta-xylosidase	2	1	-	-	1	1	-	4
EC:3.2.1.4	Cellulase	-	-	-	-	-	-	-	2
EC:3.2.1.41	Pullulanase	1	1	-	-	-	-	-	1
EC:3.2.1.45	Glucosylceramidase	1	-	-	-	-	-	-	1
EC:3.2.1.51	Alpha-L-fucosidase	1	-	3	-	-	-	-	-
EC:3.2.1.52	Beta-N-acetylhexosaminidase	-	1	4	2	2	2	2	2
EC:3.2.1.54	Cyclomaltodextrinase	-	-	1	1	1	1	1	-
EC:3.2.1.55	Alpha-N-arabinofuranosidase	3	-	-	3	2	3	3	3
EC:3.2.1.78	Mannan endo-1,4-beta-mannosidase	1	-	-	-	-	-	-	-
EC:3.2.1.86	6-phospho-beta-glucosidase	-	-	-	-	-	-	-	-
EC:3.2.1.89	Arabinogalactan endo-1,4-beta-galactosidase	-	-	-	-	-	1	1	-
EC:3.2.1.93	Trehalose-6-phosphate hydrolase	-	-	-	-	-	-	1	-
EC:3.2.1.96	Mannosyl-glycoprotein endo-beta-N-acetylglucosaminidase	-	-	-	-	-	1	1	-



Table 6 continued

EC number	Enzyme name	<i>B. dentium</i> ATCC27678	<i>B. gallicum</i> DSM20093	<i>B. longum</i> subsp. <i>longum</i> ATCC15697	<i>B. longum</i> subsp. <i>Infantis</i> ATCC55813	<i>B. longum</i> subsp. <i>Infantis</i> CCUG52486	<i>B. longum</i> subsp. <i>longum</i> DJ010A	<i>B. longum</i> subsp. <i>longum</i> NCC2705	<i>B. pseudocatenulatum</i> DSM20438
<i>Isomerases</i>									
EC:5.3.1.1	Triosephosphate isomerase	1	1	1	1	1	1	1	1
EC:5.3.1.4	L-arabinose isomerase	1	1	–	1	1	1	1	1
EC:5.3.1.5	Xylose isomerase	1	1	–	1	1	1	1	1
EC:5.3.1.6	Ribose-5-phosphate isomerase	1	1	1	1	1	1	1	1
EC:5.3.1.9	Glucose-6-phosphate isomerase	1	1	1	1	1	1	1	1

Data were obtained from sequenced *Bifidobacterium* genomes using find function in JGI-IMG; ±, present/absent; N/A no available information

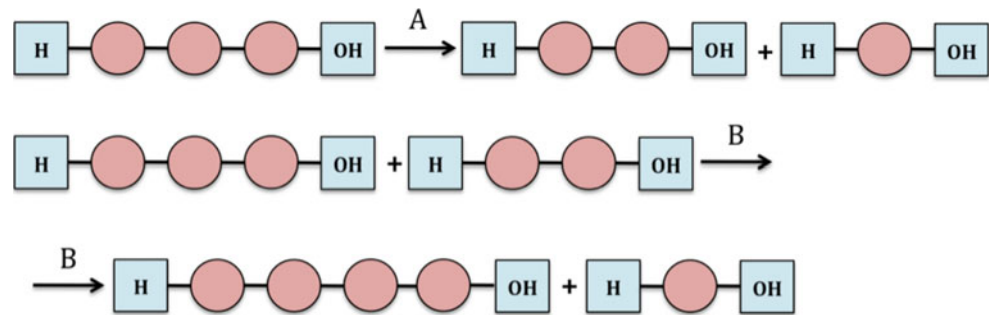
Likewise, the retaining enzymatic activity of  $\alpha$ -glucosidase, which exhibits both  $\alpha$ -glycosidic and transglycosidic activities, was shown to be commonly present in *Bifidobacterium* sp. [98, 143]. AglA and AglB from *B. adolescentis* DSM20083 have been shown to synthesize oligosaccharides from trehalose and sucrose, and maltose, sucrose and melizitose, respectively [143]. Two  $\alpha$ -glucosidases (Agl1 and Agl2) from *B. breve* UCC2003 were demonstrated to exhibit transglycosylation activity towards sugars commonly found in honey—palatinose, trehalulose, trehalose, panose and isomaltose [98].

The vast majority of available bifidobacterial genomes are predicted to encode a sucrose phosphorylase belonging to the hexosyltransferase group of enzymes (EC:2.4.1.7; Table 6). Several studies have shown that sucrose phosphorylase from *B. adolescentis* DSM20083 [144], *B. animalis* subsp. *lactis* [132] and *B. longum* subsp. *longum* [54] can hydrolyze sucrose in the presence of free phosphate with the release of glucose-1-phosphate and fructose molecules, thus bypassing the ATP-requiring step of the glucose phosphorylation/hexokinase reaction in preparation for entry into the “Bifid shunt” (Fig. 3). In addition, these characterized sucrose phosphorylases were shown to display transglycosylation activity with glucose-1-phosphate as a donor and monomeric sugars, such as D- and L-arabinose, D- and L-arabitol, and xylitol in the case of *B. longum* subsp. *longum* [144], as acceptors.

Bifidobacterial fructofuranosidases are intracellular glycosyl hydrolases involved in the hydrolysis of the  $\beta$ -2,1 glycosidic bond between glucose-fructose, and/or fructose-fructose moieties present in fructooligosaccharides, which are generally found in fruits and vegetables. Even though comparative bifidobacterial genome analysis suggests that most sequenced *Bifidobacterium* sp. encode one and in some cases two  $\beta$ -fructofuranosidases (Table 6), only a small number of such enzymes have so far been biochemically characterized, such as the  $\beta$ -fructofuranosidases produced by *B. adolescentis* [81], *B. animalis* subsp. *lactis* [26, 48], *B. breve* UCC2003 [113] and *B. longum* subsp. *infantis* [47, 157]. Most, but not all, of the characterized  $\beta$ -fructofuranosidases exhibit hydrolytic activity towards various fructooligosaccharides, sucrose and inulin.

Certain bifidobacteria have the capacity to ferment arabinofuranosyl-containing oligosaccharides derived from plant cell wall polysaccharides, such as arabinan, arabinogalactan and arabinoxytan, through the action of arabinoxytan arabinofuranohydrolases. Growth of *B. adolescentis* DSM 20083 on xylose and arabinoxytan-derived oligosaccharides was shown to induce the production of two different arabinofuranohydrolases [146]. Also different *B. longum* subsp. *longum* strains, including B667, were shown to produce arabinofuranohydrolases during growth on arabinoxytan [12, 69], although these enzymes do not appear to be a typical feature in bifidobacterial genomes.

**Fig. 3** Schematic representation of a hydrolysis reaction (a) and a transglycosylation reaction (b) performed by glycosyl hydrolases. Circles represent a sugar moiety (adapted from [141])



Nevertheless, arabinan, arabinogalactan and arabinoxyylan are considered potential prebiotics that support growth of certain bifidobacterial strains [147].

$\alpha$ -galacto-oligosaccharides derived from soymilk and galactomannan can be hydrolyzed by  $\alpha$ -galactosidases produced by *Bifidobacterium* sp. Typical  $\alpha$ -galacto-oligosaccharides, that can be utilized by bifidobacteria are the disaccharide melibiose, the trisaccharide raffinose and the tetrasaccharide stachyose. Analysis of sequenced bifidobacterial genomes suggests that the majority of bifidobacterial strains encode at least one copy of an  $\alpha$ -galactosidase-encoding gene. In spite of this,  $\alpha$ -galactosidases, capable of catalyzing hydrolysis as well as transglycosylation of  $\alpha$ -galacto-oligosaccharides, have only been studied in four bifidobacterial species, i.e. *B. adolescentis* [62, 146], *B. bifidum* [38], *B. breve* [167] and *B. longum* subsp. *longum* [31].

Starch, pullulan and amylopectin were demonstrated to be utilized by 11 different bifidobacterial strains out of 42 tested, most of which belong to the *B. breve* species [114]. This interesting observation indicates that the capacity to degrade these polymeric carbon sources may be a species-specific feature for *B. breve*. So far there are only two biochemically characterized bifidobacterial  $\alpha$ -amylase activities reported. One is produced by *B. adolescentis* Int-57 [64], while the other is represented by the ApuB enzyme produced by *B. breve* UCC2003 [87]. The latter enzyme was demonstrated to have both amylopullulanase and  $\alpha$ -amylase activities [87].

Human milk is a complex mixture of non-digestible oligosaccharides that help maintaining “healthy” gut microflora in infants (for a review see [168]). Human milk contains between 5–23 g/L of over 200 structurally different human milk oligosaccharides (HMOs) [85]. Only members of *Bifidobacterium* and *Bacteroides* were shown to be able to utilize HMOs [68]. Among the bifidobacterial species, *B. bifidum* and *B. longum* subsp. *infantis* were shown to utilize the HMOs most efficiently. In contrast, *B. breve* and *B. longum* subsp. *longum* grow on these oligosaccharides to a lesser extent, while *B. animalis* and *B. adolescentis* are incapable of degrading HMOs [66]. Interestingly, all strains from the above study were obtained from international bacterial culture collections and thus

they may have been extensively cultivated in synthetic media, which may have led to the loss of genetic traits involved in HMO utilization. The 40 kb HMO gene cluster of *B. longum* subsp. *infantis* ATCC 15697 encodes four putative glycosidases, represented by a predicted galactosidase, fucosidase, sialidase and hexosaminidase, as well as a number of transporters that are believed to be required for utilization of HMO [126]. Another study described the gene operon *lnpABCD* which encodes lacto-*N*-biose I/galacto-*N*-biose metabolic pathway responsible for the intestinal colonization of bifidobacteria through utilization of lacto-*N*-biose I from human milk oligosaccharides or galacto-*N*-biose from mucin sugars [86]. *B. bifidum*, on the other hand, is believed to produce  $\alpha$ -fucosidases and a lacto-*N*-biosidase for extracellular degradation of HMO with the concomitant release of lacto-*N*-biose, which can then be utilized intracellularly [55, 158]. *B. longum* subsp. *longum* and *B. breve* can utilize free lacto-*N*-neotetraose, one of the dominant components of HMOs, whereas *B. breve* also consumes various monomers that become available following extracellular HMO degradation [66, 158]. These different HMO utilization abilities seem to indicate niche-specific adaptation and cross-feeding abilities among various bifidobacterial species and strains.

Several recent studies have demonstrated that complex carbohydrates such as mucin, secreted by human epithelial cells, and pectin oligosaccharides, found in cell walls of plants, can also be utilized by certain *Bifidobacterium* sp. [2, 79, 92, 112].

### Carbohydrate metabolism in *B. breve* UCC2003

*B. breve* UCC2003, an isolate from nursing stool [113], is presumed to represent a typical member of the natural microbiota of an infant intestine. *B. breve* UCC2003 encodes various carbohydrate-modifying enzymes that degrade, modify or create glycosidic bonds (Table 7). Only a small number of these glycosidases are found extracellularly (e.g. the amylopullulanase [87, 114] and the endogalactanase [88, 89], while the remainder is expected to be present as cytoplasmic enzymes (e.g.  $\beta$ -fructofuranosidase

**Table 7** Characterized sugar metabolic genes in *B. breve* UCC2003

Gene/gene operon	Encoded enzyme	Substrate	Reference
<i>fos</i> operon	$\beta$ -fructofuranosidase	FOS, sucrose	[113]
<i>fru</i> operon	Enzyme EII of PTS-PEP system	Fructose	[75]
<i>apuB</i>	Amylopullulanase	Starch, amylopectin, glycogen, pullulan	[89]
<i>galA</i>	Endogalactanase	Potato galactan	[88, 89]
<i>agl1</i>	$\alpha$ -glucosidase	Panose, isomaltose, isomaltotriose, sucrose isomers	[98]
<i>agl2</i>	$\alpha$ -glucosidase	Panose, isomaltose, isomaltotriose, sucrose isomers	[98]
<i>rbs</i> operon	Ribokinase	Ribose	[97]
<i>cld</i> operon	$\beta$ -1,4-glucosidase	Cellobiose, cellodextrins	(Accepted for publication; Pokusaeva et al. 2010)

[113],  $\beta$ -1,4-glucosidase (K. Pokusaeva, M. O'Connell-Motherway, A. Zomer, J. MacSharry, G. F. Fitzgerald and D. Van Sinderen, submitted for publication),  $\alpha$ -1,6-glucosidases [98] and ribokinase [97]).

Analysis of the ribose-induced transcriptome of *B. breve* UCC2003 revealed that the *rbsACBDK* gene cluster is responsible for the metabolism of ribose, a pentose sugar that can be found in the human gut [97]. Generation and phenotypic analysis of an *rbsA* insertion mutant established that the *rbs* gene cluster is essential for ribose utilization, and that its transcription is regulated by a LacI-type regulator encoded by *rbsR*, located immediately upstream of *rbsA*. In addition, the *rbsK* gene of the *rbs* operon of *B. breve* UCC2003 was shown to specify a ribokinase (EC 2.7.1.15), which specifically directs its phosphorylating activity towards D-ribose, converting this pentose sugar to ribose-5-phosphate [97].

Moreover, transcription of the recently characterized *cldEFGC* gene cluster of *B. breve* UCC2003 was shown to be induced upon growth on cellodextrins, implicating these genes in the metabolism of carbon sources that become available upon the degradation of cellulose by other representatives of the human colon microbiota (K. Pokusaeva, M. O'Connell-Motherway, A. Zomer, J. MacSharry, G. F. Fitzgerald and D. Van Sinderen, submitted for publication). Phenotypic analysis of a *B. breve* UCC2003::*cldE* insertion mutant confirmed that the *cld* gene cluster is exclusively required for cellodextrin utilization by this bacterium. The *cldC* gene of the *cld* operon of *B. breve* UCC2003 is the first described bifidobacterial  $\beta$ -glucosidase exhibiting hydrolytic activity towards various cellodextrins.

#### Control of carbohydrate metabolism in bifidobacteria

Besides carbohydrate metabolizing activities mentioned above, a fructose PEP-PTS system, which phosphorylates fructose at the C-6 position, was identified and

characterized in *B. breve* UCC2003 [75]. This latter study also suggested that carbon catabolite control operates in this bacterium by a mechanism that appears to be different from known mechanisms. The expression level of the *rbs* operon, responsible for ribose uptake and metabolism, was shown to be decreased by approximately twofold when the culture was grown in a combination of glucose and ribose, suggesting that expression of the *rbs* operon was down-regulated to some degree by catabolite repression in the presence of a highly metabolizable sugar such as glucose [97]. Transcription of the *fos* operon, encoding  $\beta$ -fructofuranosidase from *B. breve* UCC2003, was also demonstrated to be dependent on secondary carbohydrate availability [113] as this operon was observed to be over-expressed when cells were grown on Actilight (an oligo-fructose) or sucrose, but not in the presence or combination of sucrose and glucose, or a combination of fructose and sucrose. Another interesting observation is that *B. longum* subsp. *longum* NCC2705 exhibits a lactose-over-glucose preference representing an example of reverse CCR, the mechanism of which is as yet not fully understood [96]. Selective substrate preference and different carbon catabolite control mechanisms between different bacterial as well as bifidobacterial strains indicate niche-specific adaptations between these different groups.

Many of the gene clusters involved in sugar metabolism contain LacI-type regulators, which suggest that the transcription of such gene clusters are subject to substrate-dependent repression [63, 83, 107, 108, 124, 126]. However, in just a few cases the precise action of the LacI-type regulator has been investigated in bifidobacteria [97, 132]. The RbsR protein, which controls ribose utilization through the regulation of the *rbs* operon of *B. breve* UCC2003, is presumed to act as an effective transcriptional repressor in the absence of the effector molecule, D-ribose, thereby preventing transcription [97]. Transcription of *rbs* is induced,

thus facilitating ribose metabolism, in the presence of D-ribose, which decreases the affinity of RbsR for its specific DNA operator in the promoter region of the *rbs* operon. This control mechanism is similar to RbsR regulation in *E. coli* [74]. In contrast, the binding activity RbsR from *B. subtilis* and *Corynebacterium glutamicum* does not appear to be affected by D-ribose [83, 129]. Similarly, a LacI-type regulator was identified adjacent to the *cld* operon, which was demonstrated to be involved in transport and hydrolysis of cellodextrins in *B. breve* UCC2003 (K. Pokusaeva, M.O'Connell-Motherway, J. MacSharry, G. F. Fitzgerald and D. Van Sinderen, submitted for publication).

The above findings suggest that bifidobacteria employ LacI-type repressors to control transcription of many of its genes involved in carbohydrate metabolism, but that these genes are also subject to an as yet uncharacterized CCR system. The latter system requires further research in order to expand our knowledge and understanding on sugar metabolism and its hierarchical control.

## Conclusions

Prebiotics have become part of the expanding functional food market. For development of new prebiotics and for understanding as to why certain oligo- and polysaccharides can or cannot be used as prebiotics, a better understanding about the carbohydrate-hydrolyzing enzymes encoded by *Bifidobacterium* sp. is needed. So far only a relatively small number of bifidobacterial glycosyl hydrolases have been characterized in detail [142]. The genome sequences from different *Bifidobacterium* strains has and will continue to provide us with theoretical information on putative carbohydrate—modifying enzymes and sugar transport systems, which may help these bacteria to adapt to the gastrointestinal environment and to interact with their human host. Characterization of novel glycosyl hydrolases with hydrolytic and transglycosylation activities will allow us to identify substrates that may act as novel prebiotics. More thorough research has to be performed to prove the functions of these predicted bifidobacterial enzymes and to determine their working mechanisms.

**Acknowledgments** The Alimentary Pharmabiotic Centre is a research centre funded by Science Foundation Ireland (SFI), through the Irish Government's National Development Plan. The authors and their work were supported by SFI (grant numbers 02/CE/B124 and 07/CE/B1368).

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