Ecological and molecular perspectives on responders and non-responders to probiotics and prebiotics

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Abstract

Bifidobacteria are widely used as a probiotic for their health-promoting effects. To promote their growth, bifidogenic prebiotics, including human milk oligosaccharides (HMOs), have been added to supplements and infant formula. However, the efficacy of both probiotic and prebiotic interventions is often debated, as clinical responses vary significantly by case. Here, we review clinical studies that aimed to proliferate human-residential *Bifidobacterium* (HRB) strains in the gut, and we highlight the difference between responders and non-responders to such interventions through an ecological, niche-based perspective and an examination of the prevalence of genes responsible for prebiotic assimilation in HRB genomes. We discuss the criteria necessary to better evaluate the efficacy of probiotic and prebiotic interventions and the recent therapeutic potential shown by synbiotics.

Introduction

The human gut microbiota plays an important role in human health and disease. An increasing body of work links the gut microbiota with several disease states [1–4]. As a result, therapeutic interventions aimed at regulating the gut microbiota, such as probiotics (exogenously administered microorganisms) and prebiotics (non-digestible compounds by the host that promote the growth of gut microbes), have become increasingly popular and represent increasingly marketable and growing industries [5,6]. Here, probiotics will refer to exogenously administered strains to differentiate them from autochthonous strains already residing in the gut, and the terms used in this review are summarized in Table 1. In this review, we examine the efficacy of both probiotics and prebiotics in several clinical studies from an ecological and molecular perspective. Specifically, we focus on human-residential bifidobacteria, a commonly used probiotic taxon as model organisms and the prebiotics that promote their growth.

Bifidobacteria are Gram-positive and anaerobic bacteria that were first isolated from breastfed infant feces [7], and several different species have been isolated from the gut of a variety of animals, ranging from insects to mammals [8,9]. With a few exceptions, bifidobacteria show strong exclusivity and adaptation to their specific hosts [10], and an evolutionary analysis between gut microbes and hominids has shown that bifidobacteria have cospeciated with their respective hosts over the last 15 million years [11]. We focus on bifidobacteria that naturally occur in humans (hereinafter referred to as human-residential bifidobacteria (HRB), summarized in Table 2). The type of HRB strains found varies between life stages (infant- and adult-type), suggesting that bifidobacterial adaptation to the human gut environment may be mediated by diet (host- and plant-derived glycans).

The presence of indigenous bifidobacteria is correlated with a variety of health effects,

such as the development of the immune system [12], prevention of allergy [13], and reduction of gut inflammation [14–17]. As a result, bifidobacteria are often administered as probiotics to preterm infants who are expected to lack bifidobacteria [18–21]. Additionally, there has been an increased effort to add bifidogenic prebiotics, such as HMOs, to infant formula. HMOs are resistant to pancreatic digestion [22] and act as natural prebiotics for indigenous bifidobacteria [23]. Currently, HMOs such as 2′-fucosyllactose (2′-FL), 3-fucosyllactose (3-FL), lactodifucotetraose (LDFT), lacto-*N*-tetraose (LNT), and lacto-*N*-*neo*tetraose (LN*n*T) are synthesized by engineered *Escherichia coli* strains and generally considered safe by the US Food and Drug Administration (FDA). Other prebiotics such as fructooligosaccharides (FOSs) and galactooligosaccharides (GOSs), which are enzymatically synthesized from sucrose and lactose, respectively, are also used to promote growth of bifidobacteria [24].

Despite increasing reports of health benefits conferred by probiotic bifidobacteria and bifidogenic prebiotics, studies report conflicting results, and their efficacy shows high situational variability. In certain cases, human trials using probiotic preparations are treated as a "black box," and the mechanisms that differentiate responders from non-responders to such microbiota-based therapies remain elusive. We propose that a combined perspective utilizing both an ecological framework and an understanding of molecular mechanisms is necessary to successfully implement interventions aimed at modulating the gut microbiota, with the ultimate purpose to improve health.

A combined niche- and gene-based framework

One of the long-standing issues with probiotics relates to their limited persistence in the human gastrointestinal tract. Although stable colonization is not necessarily required for

probiotics to exert benefits on host health, a substantial population must, at least transiently, establish for them to have a metabolic impact on the host and the resident gut microbiota. We propose that an applied framework based on the modern species coexistence theory could help predict colonization success, as summarized in Figure 1 [25,26]. Theory suggests that colonization outcomes are determined by a combination of niche and relative fitness differences between the exogenously administered probiotic strain (colonizer) and the indigenous baseline microbiota (autochthonous taxa) [27]. According to this framework, higher niche difference (ND) allows for niche differentiation between the invading and autochthonous taxa, reduces competition, and ultimately increases the probability of colonization success. Within the gut microbial community, differential resource specialization by gut microbes and the spatial heterogeneity within the gastrointestinal tract [28] and the mucus layer [29,30] can allow for gut microbes to reduce niche overlap. In the presence of increasing niche overlap and competition for resources, relative fitness differences (RFD) also influence the colonization outcome. As high RFD would exclude species with lower fitness in a given environment, the exogenously administered probiotic strain must be better adapted to the gut environment to colonize.

While bifidogenic prebiotics are predicted to provide a fitness advantage to bifidobacteria, the response of bifidobacteria to prebiotics varies significantly not only at the species level but also at strain level. In the prebiotics section of this review, we propose that the presence or absence of certain prebiotic-utilization genes and the strain-level genotypic diversity may explain the difference between responders and non-responders to probiotics and prebiotics. While the addition of bifidogenic prebiotics may shift the effect of ND and RFD in favor of probiotics, predicting the responder status to such therapeutic interventions requires an understanding of prebiotic consumption behavior of autochthonous/probiotic bifidobacteria at the genotype level.

In the following sections, we examine the role played by niche and fitness differences

and their impact on the colonization success of exogenously administered probiotic HRB strains (Table 3), as well as the prevalence of genes responsible for prebiotic utilization in HRB strains (Table 4). We highlight studies that examine changes in the gut microbiota composition as a result of probiotic and prebiotic interventions and show that for both probiotics and prebiotics, evaluation of efficacy can only be described within the context of the resident gut microbiota. Here, we argue that predicting the clinical responses to such interventions requires a combined understanding of the ecological and molecular mechanisms.

Probiotic Bifidobacteria

In this section, we consider individuals to be "responders" to probiotics if successful colonization of the administered strain is observed. The inability of bifidobacteria and other probiotic strains to stably colonize the adult gut microbiota is a long-standing issue in probiotic therapy [31]. Compared to the infant gut microbiota, the adult gut microbiota is more taxonomically diverse, stable [32], and resistant to colonization [33]. However, a study by Maldonado-Gómez et al. [34] using *Bifidobacterium longum* AH1206 showed that colonization is possible as long as there is niche availability (Table 3). In that study, AH1206 was orally administered to adults, and they reported long-term (at least 6 months) colonization by AH1206 in approximately 30 % of the subjects. In AH1206-responders, the baseline gut microbiota before probiotic administration had a low abundance of indigenous *B. longum* and underrepresentation in certain carbohydrate utilization genes. While AH1206 would be otherwise competitively excluded due to high RFD in favor of the autochthonous microbiota, colonization is possible if taxa that occupy the same niche (conspecifics or taxa that are superior competitors for the same resources) are absent in subjects responding to AH1206 administration. By utilizing a strain-

121 specific approach, the authors were able to show that colonization resistance in the adult gut can
122 be overcome with sufficiently high niche difference between exogenously administered probiotics
123 and the resident microbiota.

124 In contrast to the stable microbial community in the adult gut, the infant gut microbiota
125 is in rapid development. The infant gut is generally considered sterile at birth, and more open
126 niches are available during the early stages of community assembly. Earlier arriving species gain
127 an advantage due to priority effects, in which the order and timing of species arrival dictate
128 community composition (Table 1), as they colonize the available niches first [35,36]. Therefore,
129 the timing of probiotic administration becomes especially important, as seen in the administration
130 of *Bifidobacterium breve* strains in preterm infants. For example, a study by Costeloe et al. [37]
131 found that the administration of *B. breve* BBG-001 to preterm infants did not significantly reduce
132 the incidence of necrotizing enterocolitis (NEC), and colonization by bifidobacteria was not
133 observed (Table 3). In that study, the median age at the first dose was approximately 44 hours.
134 However, studies in which the first dose of *B. breve* (YIT4010 [38] and M-16V[39]) was
135 administered within a few hours of birth showed increased gut colonization by bifidobacteria
136 (Table 3). Taken together, these results suggest that the timing of arrival plays a significant role
137 in gut microbiota assembly and can influence host response to probiotic administration. Indeed,
138 when a study by Li et al. [40] compared the timing of administration of *B. breve* (strain
139 unspecified), they reported increased effectiveness when the first dose was administered a few
140 hours after birth compared to when administered more than 24 hours after birth. Furthermore, a
141 study by Horigome et al. [41] reported a decreased abundance of phylum Proteobacteria in low
142 birth weight infants given M-16V, suggesting that preemptive colonization by bifidobacteria can
143 competitively exclude undesirable taxa, and facilitative priority effects can enhance the growth
144 of other *Bifidobacterium* species. Altogether, these studies show that in the stable adult gut, the

availability of vacant niches is determined by the composition of the resident gut microbiota. On the other hand, the infant gut microbiota is in its developmental stage and niche availability is significantly affected by the timing of arrival, increasing the impact on community structure.

In addition to the timing of administration, the presence of prebiotic substances influences host response to probiotics. A study by Underwood et al. [42] using M-16V strain found that responders were fed breastmilk that contained more non-fucosylated and/or non-sialylated neutral (undecorated) HMOs, such as lacto-*N*-tetraose (LNT) (Table 3). Only a limited subset of *B. breve* strains can utilize fucosylated HMOs, while LNT assimilation ability is conserved within *B. breve* strains. Therefore, most likely, the HMOs available in the breastmilk fed to non-responder infants could not be consumed by the bifidobacteria present in those individuals. Indeed, a study by Thongaram et al. [43] showed that M-16V cannot utilize 2′-FL. These findings indicate that the fitness advantage conferred to probiotic bifidobacteria is highly dependent on the presence of HMO-utilization genes, which will be discussed in the prebiotics section of this review. A separate study administered *B. infantis* EVC001, a strain that can utilize a wide range of HMOs (both decorated and undecorated), to breastfed infants. They observed the long-term persistence of this strain in addition to a significant reduction in the amount of HMOs remaining in feces, even after the cessation of probiotic administration, suggesting that persistence was achieved through HMO consumption (Table 3) [44]. Additionally, a study by O'Brien et al. [45] reported long-term colonization by *B. infantis* EVC001 that persisted for at least one year postpartum, due to the combination of early probiotic administration and breastmilk. This is further illustrated by Duar et al. [46], who performed an *in vivo* competition experiment in two breastfed infants with two *B. infantis* strains. They found that *B. infantis* EVC001 successfully colonized the gut of both infants, and outcompeted *B. infantis* NLS, a strain that has a genetic lesion in an HMO-utilization gene cluster (Table 3). Together, these studies illustrate that prebiotics like HMOs can confer a fitness

advantage to probiotic strains and facilitate colonization in the infant gut, but only if the genes to utilize them are also present in the genomes.

Predicting responses to probiotics requires an understanding of both niche availability within the resident gut microbiota and the relative fitness of the probiotic strain. The probability of colonization success increases with high niche differences and the presence of prebiotics such as HMOs can shift RFD to favor growth of probiotics. However, prebiotic supplementation is effective only when the necessary response genes are present, and the presence of those genes is highly strain-dependent. In the following section, we focus on bifidogenic prebiotics and the prevalence of response genes in HRB strains.

Bifidogenic Prebiotics

A growing body of work provides evidence of the benefits of bifidogenic prebiotics, but the criteria that differentiate responders from non-responders has not been well understood until recently. Two studies showed that the administration of two HMOs (2′-FL and LN*n*T) to healthy infants [47] and adult patients with irritable bowel syndrome (IBS) [48] increased the abundance of bifidobacteria in some subjects, but there was no clear baseline microbiota signature that was predictive of responder status. In both instances, the baseline microbiota was profiled at the species level, and the presence of genes responsible for 2′-FL and LN*n*T assimilation could not be confirmed. We argue that species-level information alone cannot predict responder status to prebiotics, and in-depth genotype analysis of the baseline microbiota is necessary.

2-Fucosyllactose and Lacto-N-neo*tetraose*

While 2′-FL and LN*n*T have been added to commercial infant formula in several countries, the ability to utilize these two HMOs is highly strain-specific. With regards to 2′-FL, a trisaccharide comprised of lactose with an α-linked fucose residue, an ATP-binding cassette (ABC)–type transporter responsible for its uptake was identified in *B. longum* [49] and *B. breve* [50]. Further work by Sakanaka et al. [51] showed that *B. infantis* ATCC 15697^{T} possesses two functionally overlapping but distinct paralogs of this transporter (FL transporter-1 and FL transporter-2). Both paralogs import 2′-FL, although FL transporter-2 has wider substrate specificity for fucosylated HMOs. In this study, the abundance of homologs for the substrate-binding protein (SBP) of this transporter in fecal samples was positively correlated with the abundance of bifidobacteria in breastfed infants and negatively correlated with the concentration of the substrate HMOs remaining in feces. These results strongly indicate that the presence of this transporter could predict response status to 2′-FL. While the presence of this transporter is limited to the genus *Bifidobacterium*, the occurrence of this transporter varies considerably by strain (Table 4). Specifically, homologs of the FL transporter-1 SBP were found in 4 % of *B. breve* strains and 57 % of *B. infantis* strains (Table 4) [51,52]. Homologs for the FL transporter-2 SBP were more widely distributed within bifidobacteria strains, with its prevalence of 8 % of *B. breve*, 100 % of *B. kashiwanohense*, 86 % of *B. infantis*, 3 % of *B. longum*, and 13 % of *B. pseudocatenulatum* strains [51,52]. We do note that *B. bifidum* does not possess this transporter [53] as it extracellularly degrades 2′-FL to assimilate lactose, without utilizing liberated fucose [54].

LN*n*T, a tetrasaccharide comprised of galactose, *N*-acetylglucosamine, and glucose joined by β-linkages, is imported by a similar mechanism by the LN*n*T-SBP, a homolog of NahS that was first identified in *B. breve* UCC2003 [55]. The LN*n*T-SBP homolog is conserved in all analyzed strains of *B. breve* (Table 4) [52], and most strains are capable of growing on LN*n*T

[56]. About 48 % of *B. infantis* strains possess the LN*n*T-SBP homolog (Table 4).

The transporters for the utilization of 2′-FL and LN*n*T are limited to infant gut-associated species, suggesting adaptation by bifidobacteria to the breastfed infant gut environment. However, as mentioned above, their occurrence is not ubiquitous and highly strain-dependent. Therefore, in the absence of strains with the necessary transporters, individuals are unlikely to respond to such prebiotic therapies. While the addition of 2′-FL and LN*n*T to formula does provide certain benefits, not all bifidobacteria are capable of utilizing HMOs currently available in infant formula. As more types of HMOs receive approval for commercial use, the use of a wider variety of native HMO molecules with a composition that mimics natural breastmilk may further promote the efficacy of HMOs as prebiotics. In this regard, LNT could be the most effective prebiotic, as almost all infant-type HRB strains isolated to date are positive for LNT assimilation [57].

Galactooligosaccharides

Galactooligosaccharides (GOSs), which are widely used as a prebiotic, are a mixture of mono-, di-, and trigalactolyllactose (GL) species with galactose moiety(ies) attached by β-1,3/4/6-linkage(s) and produced from lactose through the transgalactosylation activity of β-galactosidases [58]. They also have been shown to effectively increase the abundance of bifidobacteria in the gut [59,60]. Furthermore, in a cohort of infants from the United States, there was a strong positive correlation between the abundance of GOS utilization genes and bifidobacteria in feces [52,61,62]. GOS utilization genes are highly conserved in HRB strains (homologs for 3′-GL utilization is highly conserved in infant type HRB strains, and homologs for 4′-GL / 6′-GL utilization genes are conserved both infant- and adult-type strains [52]) (Table

4). These results provide further support for the use of GOSs as bifidogenic prebiotics in infant formula, although its ability to selectively promote the growth of bifidobacteria is slightly lower than that of HMOs.

Lactulose

The importance of the genotypic composition of the resident gut microbiota to predict responder status to prebiotics is highlighted in a recent study by Yoshida et al. [63], which focused on lactulose. Lactulose is a disaccharide composed of galactose and fructose produced through the alkaline isomerization of lactose, is also used as a bifidogenic prebiotic [64,65]. In a previously published clinical study [65], healthy Japanese adult women were given 2 g of lactulose daily for 2 weeks, and they found that the fold change in *Bifidobacterium* abundance showed high variance (0.38 to 48.11). To examine the difference between responders and non-responders to lactulose ingestion, the study by Yoshida et al. [63] examined and identified the gene responsible for lactulose uptake by bifidobacteria, which was revealed to be a solute-binding protein (SBP), termed LT-SBP. An examination of the baseline microbiota of subjects before lactulose ingestion showed that there was a marked increase in *Bifidobacterium* in subjects with 10^7–10^9 copies of the total LT-SBP genes per gram of feces. It is interesting to note that responses to lactulose were found in individuals with a specific range of LT-SBP gene copy abundance. Changes in *Bifidobacterium* abundance were not observed in subjects who had more than 10^9 copies of LT-SBPs per gram of feces, possibly because the dose of lactulose was not sufficient to further increase *Bifidobacterium* abundance, and available niches were all occupied. On the other hand, responses were not observed in individuals with fewer than 10^7 copies per gram of feces, at which the relative abundance of lactulose-utilizing bifidobacteria

was below detection level and estimated to be < 0.001 %. This suggests that there is a minimum population size required to have a significant effect within the gut microbiota, and with an insufficient population size, lactulose-utilizing bifidobacteria may have been out-competed by other taxa with moderate ability of assimilating lactulose. Through analyzing the prevalence and abundance of bifidobacterial LT-SBP homologs in the gut microbiota (Table 4), Yoshida et al. [63] illustrate how responder status can be predicted by analyzing certain target genes within the gut microbiota.

Arabinoxylan and Type-II Arabinogalactan

Bifidobacteria also possess the genes to utilize plant-derived glycans such as arabinoxylan and arabinogalactan, and recent studies have identified the genes responsible for their utilization by bifidobacteria.

Arabinoxylan (AX) is found in cereal grains, such as wheat, rye, barley, oats, rice, and sorghum. It represents a major hemicellulose component in the cell wall of plants and is composed of a β-1,4-linked xylose polymer partially decorated with α-L-arabinofuranosyl residues at the O-2 and/or O-3 position [66]. They are further hydrolyzed into arabinoxylan hydrolysates by gut bacteria [67]. Such AX oligosaccharides have been reported to promote the growth of bifidobacteria in human clinical trials [68], but the ability to utilize them is also highly strain-dependent [69]. A study by Saito et al. [70] with *B. pseudocatenulatum* YIT 4072^{T} identified 3 transporters responsible for AX oligosaccharide utilization and found that the SBPs of these transporters have high affinities for AX oligosaccharides. Homologs of the three transporters for AX oligosaccharide utilization were mostly found in adult-type bifidobacteria

(*B. adolescentis*, *B. catenulatum* subsp. *catenulatum*, *B. longum, B. pseudocatenulatum*) [70,71], with AXBP3 being the most prevalent (22–92 % in infant type HRB, 50–100 % in adult type HRB; Table 4), indicating an adaptive response of bifidobacteria as the host diet changes from infancy to adulthood.

Type-II arabinogalactan (AG), a component of gum arabic widely used as a food additive, was shown to be bifidogenic in a clinical trial [72]. A study by Sasaki et al. [73] focused on the gum arabic arabinogalactan protein (AGP), which is preferentially utilized by specific bifidobacterial strains that possess a 3-*O*-α-D-galactosyl-α-L-arabinofuranosidase (GAfase). They isolated GAfase from *B. longum* JCM 7052 and identified it as an important genetic element in the degradation of AGP. GAfase is a cell surface-anchored enzyme that removes α-D-Galp-(1→3)- L-Ara disaccharide from AGP, and once α-D-Galp-(1→3)-L-Ara is removed, it is metabolized by type-II AG degradative enzymes [74]. However, these enzymes alone cannot act on intact gum arabic AGP in the absence of GAfase. Therefore, although type-II AG degrative enzymes are found in a wider range of bifidobacterial strains, the presence of GAfase is the predictive factor that determines responder status to gum arabic AGP. Homology searches revealed that similar to the distribution of AX oligosaccharide utilization genes, GAfase was generally conserved in adult-type bifidobacteria [73]. However, its prevalence is considerably lower (7–23 %; Table 4) than that of AX transporters, which may explain its inability to increase bifidobacterial abundance in previous studies [75].

Raffinose

Raffinose is a non-digestible oligosaccharide found in a wide variety of plants (sugar beet, sugar cane, cabbage, potato, grape, wheat, barley, corn, legumes), and a prebiotic

commercially available in Japan, the EU, and recently the US. It is comprised of galactose, glucose, and fructose residues, and the linkage between galactose and glucose is hydrolyzed by 1,6-α-galactosidase [76]. O'Connell et al. [77] identified a raffinose-binding protein (*rafB*) from *B. breve* UCC2003, and homologs of this gene were conserved in both infant- and adult-type HRB strains (Table 4), indicating its potential as a bifidogenic prebiotic.

Synbiotics

Given that prebiotics can shift RFD and ND in favor of probiotic colonization, the combination of probiotics and prebiotics, termed synbiotics, are expected to improve the efficacy of probiotic therapy. For example, a study showed that when an exogenous, porphyran-utilizing *Bacteroides* strain was administered with porphyran, a new niche was created and allowed for stable colonization [78]. Therefore, synbiotics are predicted to synergistically improve probiotic efficacy and colonization success by providing a selective fitness advantage to the probiotic strain. Two studies have tested the synergistic effect of administering *B. breve* M-16V with short-chain GOS (scGOS) and long-chain FOS (lcFOS) to two different age groups. In infants, the increase in bifidobacterial abundance was aided by the presence of scGOS and lcFOS, and a reduction in pH was also observed [79]. Synbiotics also reduced colonization by pathogens such as *Clostridioides difficile*, setting the stage for the development of a healthy gut microbial community. While the increase in bifidobacteria can be expected in infants as the gut microbiota is still developing, responses to synbiotics were also observed in young children (1–3 years old) [80]. These results show that the combined use of probiotics with the appropriate prebiotics can facilitate probiotic colonization, perhaps even in established communities, by shifting ND and RFD in favor of colonization by probiotic strains.

Conclusions

The efficacy of both probiotics and prebiotics has been recently debated. Here, we synthesized ecological and molecular perspectives that can dictate responder status to probiotic and prebiotic interventions. From an ecological viewpoint, an understanding of the relative niche and fitness difference between the exogenous probiotic strain and the diversity of the indigenous gut microbiota can help predict colonization success. Strain-level, genomic knowledge of probiotics and the baseline microbiota from a metabolic perspective can elucidate fitness differences between the two. This can then inform the selection of effective prebiotics that can promote the growth of target bifidobacteria, as well as help tailor precision therapy to better suit individual needs. Prebiotics can provide a fitness advantage to indigenous taxa that possess an adequate abundance of target genes, and also serve as a substrate for probiotics, which can then modulate the intestinal metabolic profile and ultimately influence host health. We note that further work is necessary to identify the prevalence and abundance of prebiotic-utilization genes in non-target taxa to improve the precision of prebiotic therapy. Finally, recent advances in synbiotics show that the combined use of probiotics and prebiotics are effective in improving probiotic and prebiotic efficacy. As responder status is highly individualized, a higher resolution and strain- and genotype-level analysis of both probiotics and the baseline gut microbiota would provide improved predictive power regarding responder status to probiotics and prebiotics.

Acknowledgments

This research was in part supported by JSPS KAKENHI Grant [21H02116 to T.K] and Mishima

Kaiun Memorial Foundation 2020 [to T.K.].

[81–89]

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Table 1. List of terms and definitions used in this study.

Terms	Definition
Probiotics	Live microorganisms when administered in adequate amounts, confer a health benefit on the host [81].
Prebiotics	Non--digestible compound that, through its metabolization by microorganisms in the gut, modulates the composition and/or activity of the gut microbiota, thus, conferring a beneficial physiological effect on the host [82].
Human Residential Bifidobacteria (HRB)	Bifidobacterial species that are naturally found in the human gastrointestinal tract, with two major groups (infant-type and adult-type, with some species belonging to both groups) [83].
Colonization	When a species has successfully occupied an ecological niche and persists, in which population birth rate (growth) is greater than or equal to death rate (wash-out).
Niche Difference (ND)	The difference in resource requirements of each species (high ND indicates low overlap) [27].
Relative Fitness Difference (RFD)	The difference in competitive ability and advantage between two species in a given environment [27].
Priority Effects	The effect of species arrival order on species interactions and community composition [36].
Synbiotics	A mixture of probiotics and prebiotics that benefits the host by improving the survival and implantation of live microbial dietary supplements in the gastrointestinal tract, by selectively stimulating the growth and/or by activating the metabolism target health-promoting bacteria, and thus improving host welfare [84].

Table 2. List of human residential *Bifidobacterium* (HRB) strains

Human Residential *Bifidobacterium* (HRB) Strain	HRB Type	Type Strain Isolation Source
Bifidobacterium adolescentis	Adult	Adult intestine [85]
Bifidobacterium bifidum	Adult/Infant	Infant feces [7]
Bifidobacterium breve	Infant	Infant feces [85]
Bifidobacterium catenulatum	Adult	Adult intestine [86]
Bifidobacterium catenulatum subsp. *kashiwanohense* (*B. kashiwanohense*)	Infant	Infant feces [87]
Bifidobacterium dentium	Adult (Oral)	Dental cavities [86]
Bifidobacterium gallicum	Adult	Adult intestine [88]
Bifidobacterium longum subsp. *infantis* (*B. infantis*)	Infant	Infant intestine [85]
Bifidobacterium longum subsp. *longum (B. longum)*	Adult/Infant	Adult intestine [85]
Bifidobacterium pseudocatenulatum	Adult/Infant	Infant feces [89]

Table 3. Ecological mechanisms that allow for colonization by probiotic human-residential *Bifidobacterium* (HRB) strains in the human gut.

Probiotic Strain	Clinical Conditions	Desired Clinical Response	Primary Outcome	Proposed Response Mechanism	Reference
B. longum AH1206	Adults Daily dose of 10^{10} cells of AH1206	Long-term colonization by *B. longum*	Long-term colonization observed in individuals with low abundance of *B. longum* and prevalence of certain carbohydrate utilization genes in the baseline microbiota prior to interventior	In the absence of conspecifics and competitors for the same resource, vacant niches were available for exogenously added microbes (high niche difference).	Maldonado-Gomez et al. [34]
B. breve BBG-001	Preterm infants 8.3 to 8.8 × 10^8 (CFUs) daily First dose: 44 h after birth	Colonization by bifidobacteria and prevention of infant necrotizing enterocolitis	*B. breve* colonization was not observed, and probiotic administration did not reduce the incidence of NEC.	First dose of probiotics were administered around 44 hours after birth. Pathogenic taxa may have arrived first and excluded BBG-001 (niche preemption).	Costeloe et al. [37]
B. breve YIT4010	Preterm infants 5 × 10^9 CFUs daily First dose: a few hours after birth	Colonization by bifidobacteria	Colonization by bifidobacteria observed, as well as decreased incidence of abnormal abdominal symptoms	Infants were administered YIT04010 within a few hours of birth, which allowed for colonization before the arrival of other taxa (priority effects).	Kitajima et al. [38]
B. breve M-16V	Low birth weight infants 1 × 10^9 CFUs daily First dose: ~7 h after birth	Colonization by bifidobacteria and prevention of infant necrotizing enterocolitis	Colonization by bifidobacteria observed, as well as decreased incidence of NEC	Infants were administered M-16V within a few hours of birth, which prevented the colonization of pathogenic bacteria (through inhibitory priority effects)	Satoh et al. [39]
B. breve M-16V	Low birth weight infants 1 × 10^9 CFUs daily	Colonization by bifidobacteria	Long-term colonization by *B. breve,* increased the abundance of *Bifidobacterium* species other than *B. breve* , reduced abundance of Proteobacteria	Early colonization by M-16V in the infant gut not only prevented the proliferation of pathogenic bacteria (priority effects), but also stimulated the growth of other *Bifidobacterium* species (facilitative priority effects).	Horigome et al. [41]
B. breve M-16V	Premature infants 1.66 ×10^9 CFUs once to twice daily	Colonization by *B. breve*	Higher rates of *B. breve* colonization was associated with higher consumption of undecorated HMOs in faces.	Undecorated HMOs provided a fitness advantage to M-16V and promoted its persistence (high relative fitness difference).	Underwood et al. [42]
B. breve (Strain unspecified)	Low birth weight infants 1.6 × 10^8 cells twice a day, either a few hours after birth or 24 h after birth	Formation of a bifidobacteria-rich gut microbiota	When infants given *B. breve* several hours after birth, a bifidobacteria-rich microbiota formed earlier than in infants given *B. breve* 24 h after birth	More niches were available for colonization and *B. breve* benefitted from priority effects when administered early.	Li et al. [40]
B. infantis EVC001	Infants 1.8 × 10^{10} CFUs for 21 days	Colonization by *B. infantis*	Colonization by *B. infantis* EVC001 persisted until at least 1 year postnatal due to early probiotic administration (within one month of birth), combined with breastfeeding.	The combination of early life administration and breastfeeding provided the optimum conditions for *B. infantis* EVC001, as niches were likely available and EVC001 had a fitness advantange due to presence of HMOs in breastmilk.	O'Brien et al. [45]
B. infantis EVC001	Infants 4 × 10^9 CFUs daily	Colonization by *B. infantis*	*B. infantis* EVC001 with the gene cluster that efficiently assimilates HMOs successfully colonized infant gut.	*B. infantis* with the complete HMO-utilization gene cluster had a higher fitness (than *B. infantis* with the incomplete HMO cluster and the other resident microbiota) which allowed for colonization (high relative fitness difference).	Duar et al. [46]
B. infantis EVC001	Infants 1.8 × 10^{10} CFUs daily	Colonization by *B. infantis*	Breastfed infants given EVC001 had higher abundances of bifidobacteria, and the amount of remaining HMOs significantly decreased. Reduced endotoxin production observed in groups with high abundances of bifidobacteria than in groups with low abundances of bifidobacteria.	EVC001 has higher fitness (ability to utilize wide range of HMOs) and thereby dominated the environment. Breastfeeding (HMO supplementation) allows for the strain to persist in infant gut. EVC001 reduced endotoxin production by inhibiting the growth of Gram-negative taxa by decreasing pH (production of acetate and lactate; inhibitory priority effects)	Frese et al. [44]

Table 4. Prevalence of gene sets responsible for the assimilation of prebiotics in human-residential *Bifidobacterium* (HRB) strains.

Prebiotic	Response Gene(s) (Transporter or Extracellular GH)	HRB Species	Prevalence			Homolog Search Criteria[1]	Reference
			%	Number of Strains (Hits/Searched)			
Type- II Arabinogalactan (AG)	GAfase (BLGA_00340; *B. longum* subsp. *longum* 3-*O*-α-D-galactosyl-α-L-arabinofuranosidase)	*B. adolescentis*	7%	4 /	57	Identity ≥ 60% Query coverage ≥ 60% (Subspecies not identified in analysis for *B. catenulatum* and *B. longum* groups)	Sasaki et al. [73]
		B. catenulatum group	13%	1 /	8		
		B. longum group	7%	21 /	307		
		B. pseudocatenulatum	23%	18 /	77		
Arabinoxylan Hydrolysates (AXH)	BpAXBP1 (BBPC_RS01570; *B. pseudocatenulatum* AXH binding protein 1)	*B. adolescentis*	10%	5 /	50	*Identity ≥ 60% Query coverage ≥ 60%	Saito et al. [70] Prevalence from this study
		B. catenulatum subsp. *catenulatum*	36%	4 /	8		
		B. pseudocatenulatum	62%	45 /	73		
	BpAXBP2 (BBPC_RS02280; *B. pseudocatenulatum* AXH binding protein 2)	*B. adolescentis*	4%	2 /	50	*Identity ≥ 60% Query coverage ≥ 60%	
		B. catenulatum subsp. *catenulatum*	38%	3 /	8		
		B. pseudocatenulatum	34%	25 /	73		
	BpAXBP3 (BBPC_RS02385; *B. pseudocatenulatum* AXH binding protein 3)	*B. adolescentis*	96%	48 /	50	*Identity ≥ 60% Query coverage ≥ 60%	
		B. catenulatum subsp. *catenulatum*	50%	4 /	8		
		B. catenulatum subsp. *kashiwanohense*	67%	2 /	3		
		B. dentium	100%	18 /	18		
		B. longum subsp. *longum*	50%	28 /	56		
		B. longum subsp. *infantis*	22%	4 /	18		
		B. pseudocatenulatum	92%	67 /	73		
Lactulose	LT-SBP (BL105A_0502; *B. longum* subsp. *longum* lactulose-binding protein)	*B. adolescentis*	100%	53 /	53	Identity > 65% Query coverage > 60% (Subspecies not identified in analysis for *B. catenulatum* and *B. longum* groups)	Yoshida et al. [63]
		B. bifidum	99%	94 /	95		
		B. breve	100%	108 /	108		
		B. catenulatum group	100%	16 /	16		
		B. dentium	100%	21 /	21		
		B. longum group	100%	338 /	339		
		B. pseudocatenulatum	89%	68 /	76		
2′-Fucosyllactose	FL1-BP (Blon_0343; *B. longum* subsp. *infantis* fucosyllactose-binding protein 1)	*B. breve*	4%	4 /	91	Identity ≥ 70% Query coverage ≥ 60%	Sakanaka et al. [51], Prevalence from Sakanaka et al. [52]
		B. longum subsp.*infantis*	57%	12 /	21		
	FL2-BP (Blon_2202; *B. longum* subsp. *infantis* fucosyllactose-binding protein 2)	*B. breve*	8%	7 /	91	Identity ≥ 70% Query coverage ≥ 60%	Sakanaka et al. [51], Prevalence from Sakanaka et al. [52]
		B. longum subsp. *infantis*	86%	18 /	21		
		B. catenulatum subsp. *kashiwanohense*	100%	2 /	2		
		B. longum subsp. *longum*	3%	5 /	151		
		B. pseudocatenulatum	13%	8 /	64		
LN*n*T	LN*n*T-BP (NahS) (Bbr_1554; *B. breve* lacto-*N*-*neo*tetraose-binding protein)	*B. breve*	100%	91 /	91	Identity ≥ 70% Query coverage ≥ 60%	James et al. [55] Prevalence from Sakanaka et al. [52]
		B. longum subsp. *infantis*	48%	10 /	21		
GOS 3′-Galactosyllactose	3′GL-BP (GNB/LNB-BP homolog) (BBBR_RS08090; *B. breve* 3′-galactosyllactose-binding protein)	*B. adolescentis*	2%	1 /	46	Identity ≥ 70% Query coverage ≥ 60%	Sotoya et al. [62], Prevalence from Sakanaka et al [52]
		B. bifidum	100%	60 /	60		
		B. breve	100%	91 /	91		
		B. catenulatum subsp. *kashiwanohense*	50%	1 /	2		
		B. longum subsp. *infantis*	81%	17 /	21		
		B. longum subsp. *longum*	100%	151 /	151		
		B. pseudocatenulatum	84%	54 /	64		
4′-Galactosyllactose	4′GL-BP (BBBR_RS01855; *B. breve* 4′-galactosyllactose-binding protein)	*B. adolescentis*	13%	6 /	46	Identity ≥ 70% Query coverage ≥ 60%	Shigehisa et al. [61], Prevalence from Sakanaka et al. [52]
		B. breve	97%	88 /	91		
		B. catenulatum subsp. *catenulatum*	50%	1 /	2		
		B. dentium	100%	9 /	9		
		B. longum subsp. *infantis*	14%	3 /	21		
		B. longum subsp. *longum*	87%	132 /	151		
		B. pseudocatenulatum	91%	58 /	64		
6′-Galactosyllactose	6′GL-BP (BBBR_RS02320; *B. breve* 6′-galactosyllactose-binding protein)	*B. breve*	100%	91 /	91	Identity ≥ 70% Query coverage ≥ 60%	Sotoya et al. [62], Prevalence from Sakanaka et al [52]
		B. longum subsp. *infantis*	14%	3 /	21		
		B. longum subsp. *longum*	100%	151 /	151		
Raffinose	RafB (Bbr_1867; *B. breve* raffinose-binding protein)	*B. adolescentis*	92%	46 /	50	*Identity ≥ 60% Query coverage ≥ 60%	O'Connell et al. [77] Prevalence from this study
		B. bifidum	3%	3 /	87		
		B. breve	100%	103 /	103		
		B. catenulatum subsp. *kashiwanohense*	33%	1 /	3		
		B. longum subsp. *infantis*	100%	18 /	18		
		B. longum subsp. *longum*	100%	56 /	56		
		B. pseudocatenulatum	80%	58 /	73		

a) Asterisks indicate that prevalence of homologs was reanalyzed in this study with a blastp tool against *Bifidobacterium* genomes in NCBI refseq database (https://ftp.ncbi.nlm.nih.gov/genomes/refseq/bacteria/).
When multiple sequences of the same strain from different culture collection providers were found in the database, only one representative sequence was used for analysis.

Bifidobacteria with prebiotic responder gene Bifidobacteria without prebiotic responder gene Prebiotics

Relative Fitness Difference (Probiotic vs Resident Microbiota)

High

Exogenous Probiotic > Indigenous Microbiota
(Further aided by prebiotics)

Low

Exogenous Probiotic < Indigenous Microbiota

Niche Difference

High

Niches: Open

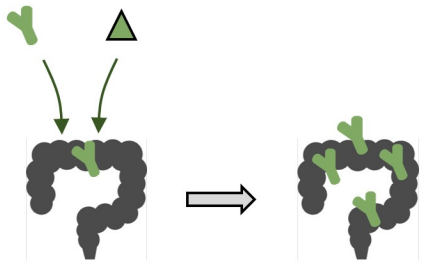
(Exogenous probiotic and indigenous microbiota competition low / resource requirement overlap low)

Low

Niches: Occupied

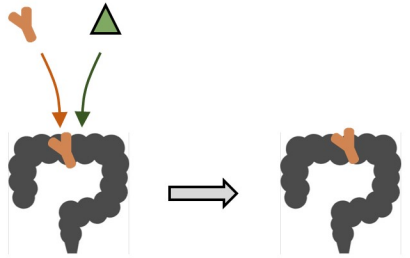
(Resource requirement overlap between exogenous probiotic and indigenous microbiota high)

a)



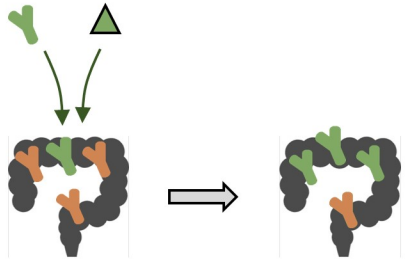
Response to probiotics and prebiotics likely. Probiotics colonize open niches and further proliferate using prebiotics.

c)



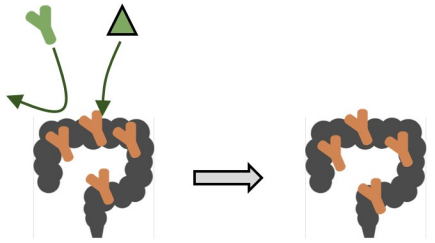
Response to probiotics possible only if niches available, as probiotics cannot outcompete resident taxa. Prebiotics cannot support growth without responder genes

b)



Response to probiotics possible if probiotics can outcompete resident taxa. Presence of prebiotics can provide competitive edge to probiotic strains.

d)



Responses to probiotics and prebiotics unlikely as niches are unavailable. Probiotics can neither utilize the prebiotics nor outcompete resident taxa.