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Gut Colonization Mechanisms of *Lactobacillus* and *Bifidobacterium*: An Argument for Personalized Designs

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Abstract

Lactobacillus and *Bifidobacterium* spp. are best understood for their applications as probiotics, which are often transient, but as commensals it is probable that stable colonization in the gut is important for their beneficial roles. Recent research suggests that the establishment and persistence of strains of *Lactobacillus* and *Bifidobacterium* in the gut are species- and strain-specific and affected by natural history, genomic adaptability, and metabolic interactions of the bacteria and the microbiome and immune aspects of the host but also regulated by diet. This provides new perspectives on the underlying molecular mechanisms. With an emphasis on host–microbe interaction, this review outlines how the characteristics of individual *Lactobacillus* and *Bifidobacterium* bacteria, the host genotype and microbiome structure,

diet, and host–microbe coadaptation during bacterial gut transition determine and influence the colonization process. The diet-tuned and personally tailored colonization can be achieved via a machine learning prediction model proposed here.

INTRODUCTION

Long-term coevolution between hosts and gut commensals has resulted in an elaborate dynamic balance that benefits both partners, and stable establishment and persistence of microbes in the gut are outcomes of this mutualism. 16S rRNA sequencing data have identified some candidate taxa (e.g., *Bifidobacterium longum*) that can stably colonize within an individual for years (Faith et al. 2013); tracing an individual strain during its gut transition provides a simplified approach to understanding host–microbe interactions at the single-strain level. Reflecting on previous research on gut colonization by bacteria, in particular *Lactobacillus* and *Bifidobacterium* spp. (**Figure 1**), we can trace a progression from human clinical data or observations from animal experiments to mechanistic findings on specific bacterial proteins or molecules mediating engraftment and on to environmental factors (such as diet or medication), host aspects (age, gender, genotype, physiology), and gut ecosystem conditions. Taking advantage of the plethora of archived bacterial genomes and advances in molecular techniques (Ahn et al. 2014, Hudak et al. 2017) (**Figure 1**), strain-level detection and surveillance under complex contexts have recently been applied to evaluating gut colonization.

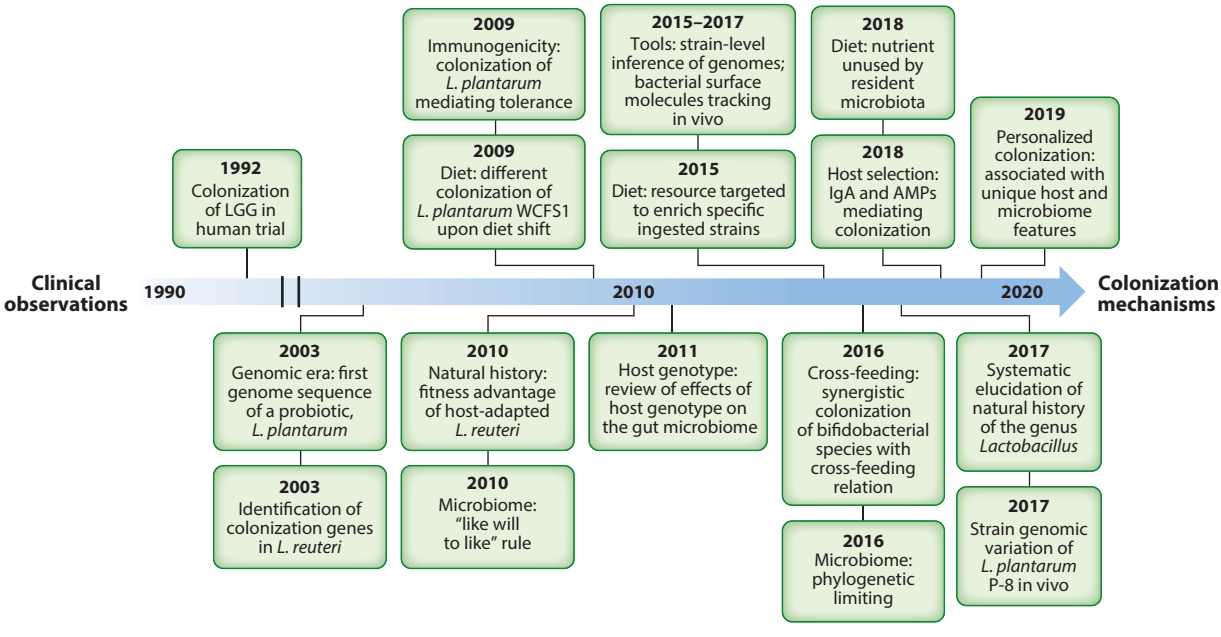

Figure 1

Timeline of selected key findings and technical advances in the history of gut colonization research by bacteria, focusing on *Lactobacillus* and *Bifidobacterium* (Ahn et al. 2014, Donaldson et al. 2018, Duar et al. 2017, Goldin et al. 1992, Hudak et al. 2017, Krumbeck et al. 2015, Maldonado-Gómez et al. 2016, Marco et al. 2009, Oh et al. 2010, Shepherd et al. 2018, Song et al. 2018, Spor et al. 2011, Stecher et al. 2010, Tang et al. 2018, Turroni et al. 2016, Van Baarlen et al. 2009, Walter et al. 2003, Zmora et al. 2018). Abbreviations: AMPs, antimicrobial proteins; IgA, immunoglobulin A; LGG, *Lactobacillus rhamnosus* GG.

These technical advances have revealed several areas of interest that may not have always been considered in earlier studies. First, the natural history of ingested strains, especially whether they are autochthonous or allochthonous, is a key evolutionary factor determining fitness (Duar et al. 2017). Second, the effects of the resident microbiome, as reflected by individualized colonization modes, underscore the two seemingly paradoxical notions of phylogenetic limiting (Maldonado-Gómez et al. 2016, Zmora et al. 2018) and phylogenetic clustering (which has been characterized as "like will to like") (Stecher et al. 2010). Third, there is evidence of host–microbe coadaptation by host-secreted molecules [immunoglobulin A (IgA)] (Donaldson et al. 2018, Joglekar et al. 2019) and antimicrobial proteins (AMPs) (Tang et al. 2018) and the in vivo genomic variation of incoming strains (Crook et al. 2019, Song et al. 2018). Fourth, metabolic interactions among ingested strains have been shown to facilitate colonization (Turroni et al. 2016). Fifth, resources targeted to enrich specific ingested strains (Krumbeck et al. 2015) and nutrients unused by resident microbiota (Shepherd et al. 2018) open a window of opportunity for diet-tuned colonization. These recent findings have been inspirational for the field of bacterial gut colonization.

In this review, we start with canonical and recent data on gut colonization, with a focus on *Lactobacillus* and *Bifidobacterium*, to provide a condensed meta-analysis of bacterial colonization diversity. Next, gene elements and key molecules in probiotics that determine and affect gut retention are summarized; the impacts of factors, such as host genotype, physiology (microbiome, age, and gender), diet, and medication history, are discussed; and the role of coadaptation is addressed, concentrating on signal pathways and immune response initiated in the host and gene and transcript shifts in bacterial strains during gut transition. Finally, we suggest that based on bacterial characteristics (such as gene features, metabolic ability, and immunogenicity) and those of the host genotype and microbiome, a personalized strategy with a specific combination of probiotics and prebiotics might more precisely modify the resident microbiome and thus contribute to long-term human health. This concept is also applicable to next-generation probiotics (O'Toole et al. 2017).

DIVERSITY OF GUT COLONIZATION MODES BY INGESTED STRAINS

The advent of high-throughput genome sequencing and bioinformatic tools has given researchers a molecular microscope with which to detect minute differences in the genetic makeup of microbial phylotypes (i.e., species and strains). The open pan-genomes of taxa at the genus (e.g., *Bifidobacterium*) (Milani et al. 2014) or species level (e.g., *Lactobacillus salivarius*) (Harris et al. 2017) indicate an abundance of species-specific and strain-specific genes. Furthermore, even in the core genome of strains of the same species, thousands of single-nucleotide polymorphism (SNP) variations are common. These differences in bacterial genomes lead us to more confidently propose that certain properties of strains, including whether they can achieve stable engraftment in the gut, may well be species- and/or strain-specific attributes.

To trace a single strain in the enteric niche, tools such as fluorescence in situ hybridization (FISH), strain-specific polymerase chain reaction (PCR), antibiotic-resistance marker tagging, and associated techniques have been developed that allow us to evaluate colonization more precisely. These tools also allow us to determine the spatial distribution of microbial strains in the gut, often termed gut biogeography.

Bacterial Establishment and Persistence in the Gut After Oral Administration

The establishment and persistence of *Lactobacillus* and *Bifidobacterium* are species- and strain-specific (**Supplemental Table 1**), as supported by results from various murine models and

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human trials. The population levels of different species and strains vary, with differences of several orders of magnitude (Frese et al. 2011) (**Supplemental Table 1**). More significant variation exists in the residence time. In animal models, the gut residence time differs significantly within *Lactobacillus johnsonii* (Denou et al. 2008) (NCC533 versus ATCC 33200) and *Lactobacillus plantarum* [WCFS1 (Marco et al. 2007) versus MA2 (Tang et al. 2016)]. Notably, *L. johnsonii* AO12 cannot initiate gut colonization (Geva-Zatorsky et al. 2017). Well-controlled clinical data provide further evidence of such colonization discrepancies. The persistence periods of *Lactobacillus rhamnosus* strains (GG, LC705, and DR20) after administration ceased to differ significantly (Kankainen et al. 2009, Tannock et al. 2000).

Although different species and strains exhibit diverse engraftment results in the gut, colonization of most probiotics in adults is short term, with only a limited number of exceptions (such as *B. longum* AH1206) (Maldonado-Gómez et al. 2016), and the individual colonization modes of persisters and nonpersisters have been demonstrated. Probiotic intervention at early stages of life is more likely to engraft (Gueimonde et al. 2006, Schultz et al. 2004) (**Supplemental Table 1**), presumably as a result of niche preemption. Similarly, antibiotic treatment can result in longer persistence times, possibly because of phylotype vacancies or easier invasion into a disturbed microbiome (Denou et al. 2008). Host-adapted strains are more likely to achieve fitness advantages, as evidenced by the host origin–specific colonization of *Lactobacillus reuteri* strains (Frese et al. 2011). In addition, the establishment and persistence of incoming bacteria have been shown to vary significantly with host genetic backgrounds (Ganesh et al. 2018; Marco et al. 2007, 2009; Zhou et al. 2019) and physiological status [e.g., gender (Frese et al. 2011) and microbiome (Grimm et al. 2015)]. This suggests that we should be cautious when comparing data from different studies, considering the possible differences in dose; administration frequency and activity of the strain(s); detection threshold of various tools; and host diet, genotype, and physiology.

Gut Biogeography

The gut biogeography of gut symbionts is also diverse, although this field, especially the spatial distribution of probiotics along the transverse axis of the intestines (the gut lumen, colonic mucus layers, and colonic crypts), has received little attention. However, gut biogeography has been extensively studied for Bacteroidetes (Donaldson et al. 2016) and segmented filamentous bacteria (SFB) (Atarashi et al. 2015). SFB, a strong Th17 inducer, resides largely in the ileum and tightly adheres to the small intestinal epithelium (Atarashi et al. 2015), whereas *Bacteroides fragilis*, which penetrates the colonic mucus of the host and functions as a Treg inducer, is located in colonic crypts (Lee et al. 2013). For probiotics, *Bifidobacterium adolescentis* L2-32 was shown to have a similar colonization mode to SFB; i.e., it attaches closely to the ileal epithelium (Tan et al. 2016). *Lactobacillus farciminis* favors the ileum niche to the colon (Da Silva et al. 2015), whereas *L. reuteri* 100-23 forms a biofilm in the mouse forestomach (Frese et al. 2013).

GUT COLONIZATION MECHANISMS OF *BIFIDOBACTERIUM* AND *LACTOBACILLUS*

Considering bacterial colonization mechanisms from an ecological perspective, both the "seed" (the bacterial strain) and the "soil" (the gut ecosystem conditions) are important. Successful establishment requires that strains have the ability to resist the harsh gut environment, possess suitable surface architecture to attach to the intestinal epithelium or mucus, produce metabolic molecules to mediate the colonization resistance of other gut competitors, manage nutrient availability to ensure proliferation, invoke mild immunogenicity to establish immune tolerance, promote

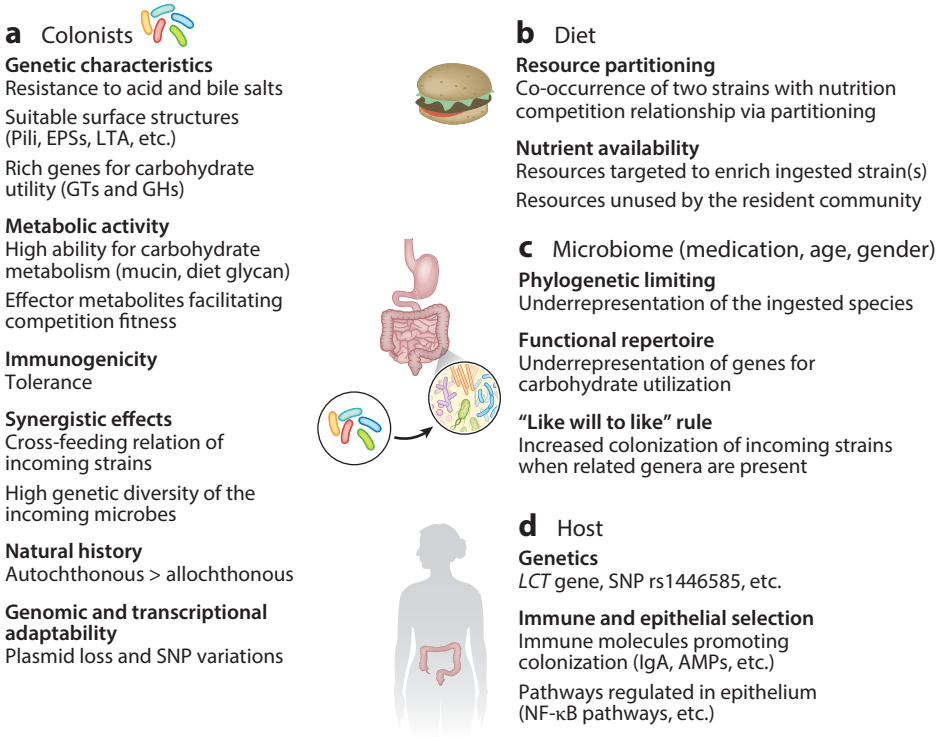

Figure 2

Criteria needed to achieve stable colonization by ingested bacteria. Gut colonization by ingested strains can be determined by four factors. (*a*) Specific genomic background, active metabolic capability, appropriate synergistic effects with other incoming strains, natural history, and genomic and transcriptional adaptability during gut transition all contribute to a good colonist. (*b*) Diet, in terms of resource partitioning and nutrient availability, is also important. (*c*) The host microbiome can exert phylogenetic and functional selection on ingested bacteria. (*d*) Host genetics and physiology also affect gut colonization by engrafted strains. Abbreviations: AMPs, antimicrobial proteins; EPSs, exopolysaccharides; GHs, glycoside hydrolases; GTs, glycosyltransferases; IgA, immunoglobulin A; LTA, lipoteichoic acid; SNP, single-nucleotide polymorphism.

efficient cell–cell communication to form a symbiotic relationship, and mediate harmonious cross talk with the host (host selection and microbiome) (**Figure 2**).

Some genes and molecules of *Lactobacillus* and *Bifidobacterium* that mediate host–microbe interactions have been identified via sequence homology with validated colonization genes in pathogens; identification of genes upregulated during gut transition; comparative genome analysis among strains with different colonization phenotypes; and recognition of the importance of biofilm forming [e.g., exopolysaccharides (EPSs)] and quorum sensing (e.g., *luxS*) in host adhesion. As few previous studies or reviews have provided a summary of molecules or genes that function in the colonization of the gut by probiotics, we have compiled a list of genes that play a role, validated in vivo, in the persistence of *Lactobacillus* and *Bifidobacterium* (**Supplemental Table 2**).

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Proposed Characteristics of Good Colonists

Bacterial adaptation to the gut microenvironment, microbial molecules that mediate host interaction, synergy among incoming strains, and the natural history of bacteria should all be taken into consideration when seeking good colonists.

Bacterial adaptation to the gut microenvironment. Bacterial adaptation to the gut microenvironment, in terms of metabolic activity, resistance to bile salts and acid, immunogenicity, and genomic and transcriptional adaptation during gut transition, is essential for their establishment and persistence in the gut.

Carbohydrate utilization. Carbohydrate metabolism provides gut commensals with a carbon source and energy, promoting bacterial establishment and survival in the gut. The available carbohydrates in the human enteric environment include diet-derived components, human milk oligosaccharides (HMOs), and host-secreted mucus glycoproteins (mucins). Nearly 14% of the annotated genes in *Bifidobacterium* encode enzymes involved in carbohydrate metabolism (Milani et al. 2014), and the frequent distribution of various glycoside hydrolase (GH) genes among different species has been well described (Milani et al. 2015). This important metabolic characteristic, especially when targeted at HMOs, endows bifidobacteria with gut colonization advantages as pioneer colonists in early human life. Host-derived mucin glycan foraging has been reported in limited numbers of phylotypes, including *Bifidobacterium*, *Akkermansia muciniphila*, and *Bacteroides*. Within the bifidobacterial species, members of *B. longum* subsp. *longum*, *B. longum* subsp. *infantis*, and *Bifidobacterium breve* have been shown to degrade mucin (as reviewed in Tailford et al. 2015). *Bifidobacterium bifidum* PRL2010 showed the ability to utilize O-linked glycans in mucin, which is believed to be an important colonization factor. The gene repertoire responsible for such host-derived glycan catabolism is conserved in *B. bifidum* (Turroni et al. 2010), indicating a fascinating example of host–microbe coevolution.

Supplemental Material >

The greater ease of genetic manipulation of many strains of *Lactobacillus*, compared with the relatively intractable *Bifidobacterium*, has provided clearer molecular evidence for this genus (**Supplemental Table 2**). Inactivation of genes involved in carbohydrate metabolism in *L. reuteri* 100-23 (Sims et al. 2011, Tannock et al. 2012), *Lactobacillus acidophilus* NCK1909 (Goh & Klaenhammer 2014), and *L. johnsonii* NCC533 (Denou et al. 2008) resulted in a significant reduction in strain colonization activity.

Resistance to bile salts and acid. The survival of digested bacteria is a key factor in their establishment in the gut, including bacterial resistance against the bile salts and acid encountered in the enteric environment. In *L. reuteri* 100-23, the absence of the *ureC* gene, which encodes a protein involved in acid resistance, significantly reduced its ecological fitness (Krumbeck et al. 2016). Expressing the listerial betaine uptake system (*BetL*) in *B. breve* UCC2003 markedly improved its tolerance to gastric juices and resulted in increased colonization levels (Sheehan et al. 2007). Similarly, heterologous expression of bile salt hydrolase genes (*bshA* from *L. acidophilus* NCFM and *bshB* from *L. johnsonii* NCK88) in *Escherichia coli* C600 significantly increased colonization biomass in the feces of germ-free mice compared with that of control *E. coli* C600 (Dimarzio 2016).

Immunogenicity. The immune system in the gut exerts a barrier function by distinguishing harmful microbes and antigens from commensals and appropriate antigens (e.g., dietary components). Gut-associated lymphoid tissue (e.g., Peyer's patch), microfold cells (M cells), and antigen-presenting cells (e.g., macrophages and dendritic cells) cooperate to sample, transcytose, and recognize microbes and antigens in the gut lumen (**Figure 3**). Therefore, to avoid eradication by the gut immune response, probiotic strains are often characterized by mild (nonproinflammatory) immunoregulatory effects. *B. adolescentis* L2-32 was reported to induce Th17 cells at a level comparable with SFB in an immune-tolerant manner, without affecting Th1 cell levels, inducing inflammation-associated immune cell subtypes, or provoking drastic transcriptional upregulation of genes associated with pathogenic Th17 cells, and without producing abnormal histological

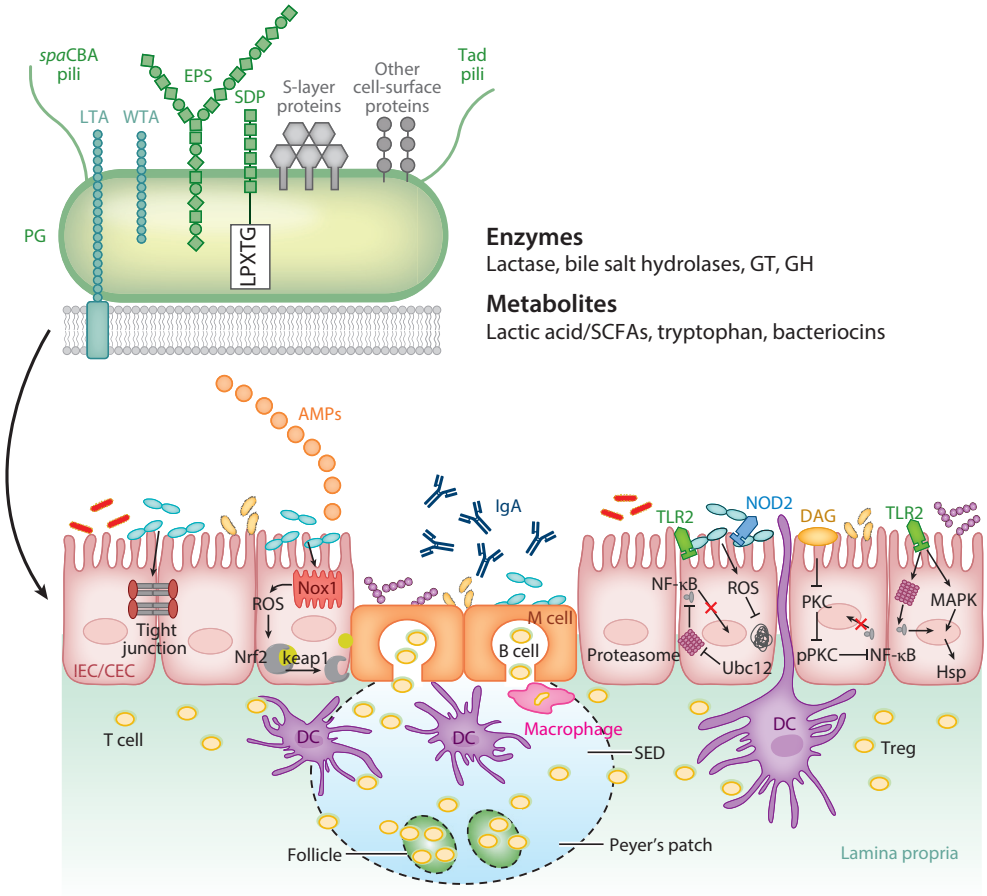

Figure 3

Cross talk of host immune and epithelial cells with ingested *Lactobacillus* and *Bifidobacterium* strains, with an emphasis on bacterial surface structures and effector molecules, and selected examples of host immune and epithelial response. Abbreviations: AMPs, antimicrobial proteins; CEC, colonic epithelial cell; DC, dendritic cell; EPS, exopolysaccharide; GH, glycoside hydrolase; GT, glycosyltransferase; IEC, intestinal epithelial cell; IgA, immunoglobulin A; LTA, lipoteichoic acid; MAPK, mitogen-activated protein kinase; PG, peptidoglycan; PKC, protein kinase C; ROS, reactive oxygen species; SCFAs, short-chain fatty acids; SDP, sortase-dependent protein; SED, subepithelial dome; WTA, wall teichoic acid.

signs (Tan et al. 2016). Oral administration of *L. plantarum* cells induced genes that are essential for mediation of the appropriate immune responses in the duodenum of healthy adult humans without provoking coordinated induction of inflammation-related key genes and without observable infiltration of immune cells (Van Baarlen et al. 2009). Studies of bacterial immunogenicity should consider synergy and counteraction effects among ingested strains and their interaction with microbiota (Christensen et al. 2002).

Gene and transcript shift. Variation in the gene expression of ingested bacteria occurs during bacterial inoculation, transition, and persistence in the gut. *L. plantarum* (Marco et al. 2007, 2009) and *L. johnsonii* (Denou et al. 2007) exhibit active transcription during gut transition. In fact, incoming *L. plantarum* WCFS1 can differentially regulate genes involved in cell surface–related functions [e.g., lipoteichoic acid (LTA)]. Notably, such gut adaptation of *Lactobacillus* strains is gut-segment

specific (Denou et al. 2007, Marco et al. 2007). Gene loss and point mutation can also occur under gut selection pressure (Crook et al. 2019, Song et al. 2018, Zhao et al. 2019). For example, *L. plantarum* P-8 demonstrated reductive evolution characterized by frequently losing plasmids upon gut coadaptation with the host (Song et al. 2018). Each individual strain showed SNP variations after gut transition when a cocktail of 12 strains of different *Lactobacillus* and *Bifidobacterium* was fed to germ-free mice (Y. Xiao, J. Zhao, H. Zhang, Q. Zhai & W. Chen, unpublished results).

Microbial molecules that mediate host interaction. The bacterial molecules that mediate and affect colonization can be divided into two groups: intestine-anchored structures and metabolites that provide competitive advantages in niche occupation (**Figure 3**).

Intestinal tissue–anchored/adhesion architectures. Cumulative evidence ranging from in vitro adhesion affinity to in vivo molecular interactions indicates that suitable surface structures, including pili [tight adherence (Tad) pili or *spa*CBA (secreted LPXTG-like) pili], EPSs, LTA, and surface-layer (S-layer) proteins, facilitate host–microbe interactions (**Figure 3**). The pili gene *SpaC* was shown to be essential for human intestinal mucus binding of *Lactobacillus rhamnosus* GG (LGG) (Kankainen et al. 2009), whereas the inactivation of a gene-encoding S-layer protein A (SlpA) in *L. acidophilus* NCFM resulted in reduced adhesion to Caco-2 cells and altered its cell morphology (Buck et al. 2005). EPS-producing strains can form a thick biofilm at the interface of gut tissues and lumen to limit the effect of intestinal mucus and epithelial turnover and help avoid being washed out, thereby contributing to bacterial persistence. The function of EPSs in in vivo bacterial colonization has been validated in *B. breve* UCC2003 (Fanning et al. 2012) and *L. reuteri* strains (Sims et al. 2011, Walter et al. 2008). LTA is another known microbe-associated molecular pattern that interacts with host TLR2 and TLR6, ensuring the in vitro immunoregulatory function of LGG (Claes et al. 2012) and markedly affecting the in vivo colonization ability of *L. reuteri* 100-23 (Walter et al. 2007) and *Lactobacillus casei* (Licandro-Seraut et al. 2014). Additional evidence for the effects of such molecules on bacterial ecological fitness is listed in **Supplemental Table 2**.

Supplemental Material >

Recently, precise tools have been developed to trace the surface molecules of bacterial strains, including peptidoglycan, capsular polysaccharides, and lipopolysaccharides (LPSs), which makes it possible to observe and record the interaction dynamics of bacterial structures with specific cell types and receptors in the human intestine (Geva-Zatorsky et al. 2015, Hudak et al. 2017). Structural analyses of bacterial molecules that mediate host interactions (Sequeira et al. 2018) and modification of the bacterial surface architecture (Turroni et al. 2013) to enhance the potential gut colonization activity of strains via engineered tools have also been reported.

Effector metabolites: bacteriocins, lactic acid, short-chain fatty acids, and tryptophan. Ingested strains can achieve a colonization advantage via metabolites that accelerate colonization resistance against competitors in the gut. The survival and growth inhibition of adherent-invasive *E. coli* (AIEC) by *L. rhamnosus* GG and *L. reuteri* 1063 were reported to be correlated with lactic acid and reuterin levels (Van den Abbeele et al. 2016). *B. longum* can exert gut epithelial cell protection against *E. coli* O157 infection via produced acetate (Fukuda et al. 2011), whereas *Bacteroides* species mediate colonization resistance against *Salmonella* infection directly by producing propionate rather than by modifying host immune pathways (Jacobson et al. 2018). Bacteriocin Abp118 released by *L. salivarius* UCC118 is a direct mediator against infection of *Listeria monocytogenes*, as inactivation of the gene *abp118* or endowment of pathogens with immunity specifically against the bacteriocin caused ineffective protection (Corr et al. 2007). Although precisely what effect these antimicrobial molecules produced by ingested strains have on individual commensals

(rather than pathogens) is still poorly understood, similar conclusions can be reached via a shared rationale. For example, it has been shown that bacteriocin production by enterococci mediates niche competition in the mammalian gastrointestinal tract (Kommineni et al. 2015). Meanwhile, the alteration of fecal metabolite profiles after strain administration and concurrent variation in colonized gut microbiota might provide indirect evidence.

Other metabolites, although without observable antimicrobial effects, are tightly connected with the host immune system and therefore have the potential to influence bacterial colonization. The tryptophan catabolites (indole derivatives) produced by *L. reuteri* were shown to promote differentiation of $CD4^+$ T cells into immunoregulatory T cells and induce IL-12 (Zelante et al. 2013) via activation of the aryl hydrocarbon receptor (Cervantes-Barragan et al. 2017).

Synergy among incoming strains. Cell–cell communication (quorum sensing) is considered important for strain colonization. Inactivation of *luxS* in *L. reuteri* 100-23C, which is involved in the biosynthesis of the signaling molecules autoinducer-2 (AI-2) and AI-3, caused an increase in the thickness of the biofilm formed in vivo and affected its ecological performance (Tannock et al. 2005). Inhibition of the production of AI-2 in *B. breve* UCC2003 significantly reduced its persistence in the murine gut, although the contribution of *luxS* to colonization is presumably associated with bacterial iron acquisition rather than biofilm formation (Christiaen et al. 2014). A link was recently reported between quorum sensing and sugar metabolism in *E. coli* (Ha et al. 2018). Regardless of the mechanisms involved, these observations indicate that cell–cell communication plays a role in the ecological performance of *Lactobacillus* and *Bifidobacterium* in the gut.

To improve fitness in the gut environment, strains may switch off the expression of, or even lose, some genes that are not strictly required. This can avoid the energy consumption associated with function maintenance but may lead to a reliance on others in the community for these lost functions. The cross-feeding behavior observed among gut commensals illustrates such an interdependency, with the genus *Bifidobacterium* serving as a prototype. Various bifidobacterial species with complementary metabolic abilities have been reported to not only cooperate in the use of complex carbohydrates in vitro (Rivière et al. 2018) but also enhance the in vivo persistence levels of each strain when they co-occur (Turroni et al. 2016). In addition to mutualism within the genus, bifidobacterial strains can expand the availability of carbohydrates for other gut symbionts such as members of Bacteroidetes (Sonnenburg et al. 2006, Turroni et al. 2016).

High diversity and plasticity of genotypes and phenotypes of ingested microbes might also be important for successful establishment and adaptation in the gut. For example, it has been shown that fecal microbiota transplantation (FMT) can result in the engraftment of a large subset of strains (Smillie et al. 2018) and modulate the resident microbiome more efficiently than a specific probiotic strain or probiotic consortia.

These findings suggest that probiotic consortia rather than single strains might be better colonists. The metabolic interactions, the higher diversity of ingested strains, and their compatibility with the microbiome should be considered if gut colonization is the aim.

Evolution and natural history of *Lactobacillus* and *Bifidobacterium*. The majority of current studies of the gut colonization of strains are based on experimental settings that are divorced from the natural history of the bacterium. Most studies are conducted with allochthonous strains (e.g., those isolated from plants or fermented foods and then studied in humans) and thus cannot be expected to perform well in their new ecosystem. In addition, many strains possess resilience in environmental niches, making it difficult to identify their true ecosystems. Our increased access to phylogenomic analyses of probiotic and metagenomic data sets presents an opportunity to further study this concept.

A model that describes the genus *Lactobacillus* as evolving from free-living to nomadic and finally host-adapted has been proposed, and species within this genus have been categorized into these three stages (Duar et al. 2017). We go further by analyzing bacterial population behavior instead of focusing only on reference strains of individual *Lactobacillus* species, confirming the lifestyle transition mode of *Lactobacillus* and elucidating the host-adapted nature of *Bifidobacterium* (Xiao et al. 2020). It is reasonable to assume that using host-adapted strains achieves higher levels of ecological performance, is more likely to exert beneficial functions that facilitate host fitness, and promotes an appropriate (tolerant) interaction with the host immune system. Some studies support this concept. The majority of commercial probiotics, which only colonize the human gut transiently, are allochthonous, whereas the probable autochthonous strain *B. longum* AH1206, which belongs to a species of the human core gut microbiome, exhibited long-term persistence in a subset of subjects (**Supplemental Table 1**) (Maldonado-Gómez et al. 2016). In addition, *L. reuteri* from rodents can achieve more successful colonization in mice compared with those from other hosts (**Supplemental Table 1**) (Frese et al. 2011).

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Although research on the natural history of the genus *Bifidobacterium* is limited, the phylogenomic analysis also indicates a metabolic conservatism that may account for gut colonization. Core genes shared by members of a given phylogenetic unit, which are believed to be inherited from common ancestry, can provide fitness advantages to its members in their natural ecosystems. All 13 enzymes involved in Bif shunt pathways of *Bifidobacterium* are among a set of approximately 480 core genes of all *Bifidobacterium* species, and an unrooted dendrogram based on Xfp, one of these enzymes, can distinguish the *Bifidobacterium* group from other bacterial phyla, suggesting the ecological conservatism of metabolic functions within the genus (Sanders et al. 2018).

Diet

Beyond the often observed diet-induced variation in the composition and abundance of specific groups of gut commensals (Lewis et al. 2015, Wu et al. 2011), we can now provide further evidence in this field by observing bacterial responses to diet at the single-strain level.

A polysaccharide-rich diet significantly elevated the population levels of ingested *L. plantarum* WCFS1 in mice compared with a prototypic Western diet (Marco et al. 2009), and *B. longum* 274 could persist in the murine gut under a polysaccharide-rich diet for at least one month during the washout period, whereas it could not initiate colonization under the Western diet (Y. Xiao, J. Zhao, H. Zhang, Q. Zhai & W. Chen, unpublished results). Dietary prebiotics altered the abundances of five indigenous *Bifidobacterium pseudocatenulatum* strains to different extents that could be linked to differences in gene functions associated with carbohydrate metabolism (Wu et al. 2017). Interestingly, two nutrient competitors (*L. reuteri* strain 100-23 and *L. johnsonii* strain 100-33) can achieve cohabitation via resource partitioning (Tannock et al. 2012). Apart from carbohydrates, higher exposure to dietary tryptophan caused selective expansion of *L. reuteri* rather than *L. johnsonii* in IDO1 knockout mice (the Trp metabolism by IDO1$^{-/-}$ mice was largely interrupted, which resulted in increased availability of Trp in the gut) (Zelante et al. 2013).

Two strategies have been designed to use diet interventions to facilitate the colonization by specific ingested strains. One is to use resources targeted at enriching the incoming strain(s) (**Figure 4*a***). An in vivo selection (IVS) model was developed to enrich specific groups of gut microbiota by feeding prebiotics [e.g., galactooligosaccharides (GOSs)] to human subjects and using a combination of an enriched *B. adolescentis* isolate and GOSs to enhance the colonization ability of bacterial strains in rats (Krumbeck et al. 2015). However, the abundance of *B. adolescentis* IVS-1 was not enhanced by GOSs in humans (Krumbeck et al. 2018). Another human trial indicated that a combination of *L. reuteri* DSM 17938 and prebiotics enhanced the metabolic activity

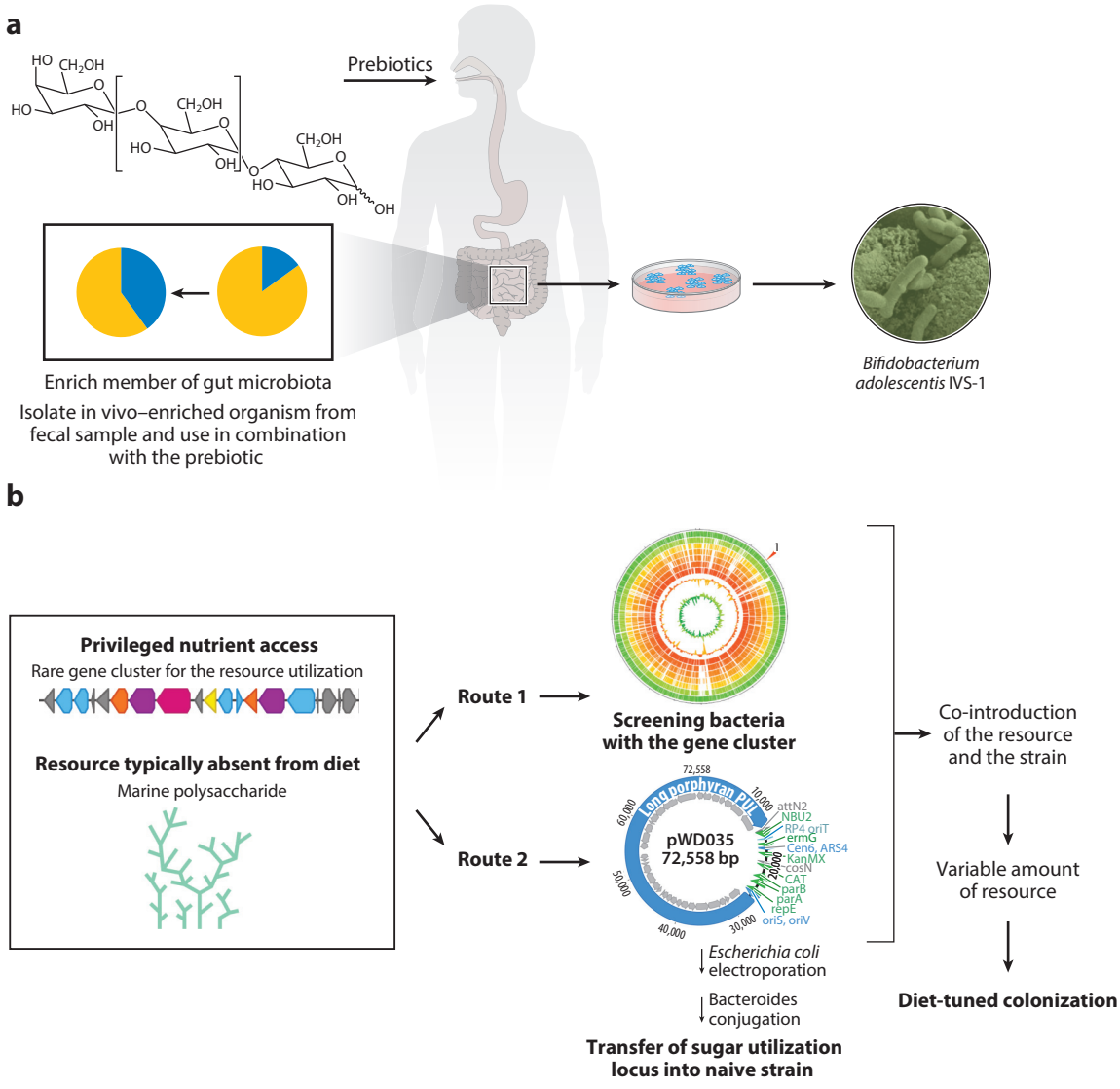

Figure 4

Diet strategies with the potentials to facilitate gut colonization by incoming strains. (*a*) In vivo selection (IVS) model using an optimized combination of prebiotics and ingested probiotics. (*b*) Strategy that utilizes resources unused by the resident microbial community. Panel *a* adapted with permission from Krumbeck et al. (2015); copyright 2015 American Chemical Society.

of the strain, but it did not increase fecal population levels or persistence (Rattanaprasert et al. 2014). Our clinical trial indicated that GOSs could enrich the population level of *B. pseudocatenulatum* in the human gut, whereas fructooligosaccharides (FOSs) elevated biomass of *B. adolescentis* (Y. Xiao, J. Zhao, H. Zhang, Q. Zhai & W. Chen, unpublished results). It should be mentioned that such specific abundance enhancement is also individualized. More clinical trials are required to evaluate the synergism of probiotics and prebiotics, and dose effects should be considered.

The other strategy is to utilize resources unused by the resident microbial community (**Figure 4*b***). *Bacteroides ovatus* (NB001), with a rare gene cluster involved in porphyran (a

marine polysaccharide) utilization, can be stably established under the administration of porphyran, irrespective of differences in gut microbiota (Shepherd et al. 2018). Furthermore, this privileged resource access can overcome the competitive exclusion of a previously introduced isogenic strain [without porphyran polysaccharide utilization locus (PUL)], and its resident biomass can be tuned with the dosage of porphyran. Similarly, *Bacteroides plebeius* DSM 17135 enjoys exclusive access to seaweed (a resource typically absent from the diet), and cointroduction of this resource with DSM 17135 increased the abundance of the strain in the short term (Kearney et al. 2018). However, this strategy has not been applied to members of *Lactobacillus* or *Bifidobacterium*.

Host Aspects

Given the amount of interpersonal variation in gut bacterial communities and the huge number of genetic loci that can potentially provide feedback to impact colonized bacteria, it is likely that some strains can rapidly establish and stably engraft in some subjects but cannot colonize in others.

Host genetics. Host genotype variation in some gene loci has been associated with differences in the abundances of colonized bacterial groups in the gut (as reviewed in Kurilshikov et al. 2017). The *Bifidobacterium* in the gut is associated with the *LCT* gene and SNP *rs1446585* of the host, which reflects lactose intolerance. The microbial quantitative trait loci are also found for *Lactobacillus*. More direct evidence is that gene modification in mice increased the population level of ingested *B. breve* UCC2003 (EPS−) (Fanning et al. 2012), and even the presence or absence of the maternal gene *FUT2* can affect the establishment of a bifidobacteria-laden microbiota in their infants (Lewis et al. 2015).

Immune selection: immunoglobulin A and antimicrobial proteins. IgA is the most abundant antibody in the human body. The majority of IgA appears in the gut, where it has been shown to function as a part of host immune defense, targeting pathogens and pathobionts (such as colitogenic bacteria) by coating the bacterial surface (Palm et al. 2014). IgA coating is not constrained to pathogens and is common among gut commensals, indicating it is a stable-state intervention in gut persisters. Gut commensals are also directly regulated by epithelial AMPs (Gallo & Hooper 2012).

Species-level and strain-level IgA coating has been found in members of *Lactobacillus* and *Bifidobacterium* (Geva-Zatorsky et al. 2017); notably, *Lactobacillus* was among the top four genera found to be enriched in the IgA+ fraction of gut bacteria in specific-pathogen-free mice (Palm et al. 2014). IgA not only promotes the adherence of commensal bacteria to intestinal cultures in vitro (Mathias et al. 2010) but also facilitates surface coating of *B. fragilis* to initiate specific immune recognition to promote mucosal colonization (Donaldson et al. 2018). Furthermore, *Bacteroides thetaiotaomicron* binds 7-6IgA (heavily glycosylated IgA) via a surface LPS structure, which can induce the expression of mucus-associated functional factor (MAFF) of the strain and provide colonization advantage in the gut (Nakajima et al. 2018). Other evidence is that microbe-specific IgA induced by *B. thetaiotaomicron* can regulate expression of a fructan PUL of the strain, affecting the ability of the strain to colonize the gut by modulating metabolism of dietary fructan (Joglekar et al. 2019). However, an opposite mechanism has also been demonstrated in which efficient colonization requires evasion of B-cell responses and inhibition of Ig profile induction. *B. breve* UCC2003 (EPS−) showed greater colonization activity in B cell–deficient mice than in wild-type control mice, and the poor colonist *B. breve* UCC2003 (EPS−) induced higher total fecal IgA levels than did *B. breve* UCC2003 (EPS+) (Fanning et al. 2012). The mechanism by which IgA exerts such seemingly divergent effects requires further elucidation, and the function of IgA coating in gut colonization by *Lactobacillus* and *Bifidobacterium* remains a mystery.

For some gut commensals, AMPs but not IgA have been shown to be involved in colonization. Il17F$^{-/-}$ mice showed increased abundances of the Treg-inducing group *Clostridium* cluster XIVa, which was shown to be caused by decreased expression of two AMPs, Ang4 and PLA2 (Tang et al. 2018). Whether these AMPs have similar effects on the ecological performance of other Treg-inducing probiotic strains requires further study. In addition, two host adaptive immune routes—the major histocompatibility complex (MHC)-dependent adaptive immune mechanisms (e.g., SFBs, *Coprococcus*, *Holdemania*, and *Mucispirillum*) and MHC-independent mechanisms (*Dehalobacterium*)—that influence the abundance of specific members of host microbiota have been summarized by Davenport (2020). The function of these pathways on gut colonization by *Lactobacillus* and *Bifidobacterium* needs further investigation.

Epithelial cell response. The gut physical barrier and local immunity provide two defense routes for antigens and microbes, which mainly involve effector molecules, immune cells (e.g., dendritic cells), epithelial cells (small intestinal and colonic compartments), cell receptors, and signaling molecules. LGG (Lin et al. 2009), *L. plantarum* cells in the mid-log phase (Van Baarlen et al. 2009), and *L. reuteri* (Ganesh et al. 2018) have been shown to inhibit NF-κB activation in epithelia, which might be due to enhanced production of reactive oxygen species to inactivate the key regulatory enzyme Ubc12 or the inhibition of upstream protein kinase C phosphorylation (pPKC)-mediated signaling. *Bifidobacterium lactis* strain BB12 exhibited an opposite mechanism, transiently inducing NF-κB and mitogen-activated protein kinase signaling both in vitro and in vivo in a TLR2 receptor-dependent manner (Ruiz et al. 2005). A mixture of three *Lactobacillus* strains was shown to not only regulate dendritic cells to produce cytokines after recognition by TLR2 and NOD2 receptors but also exert protection on the tight junctions (ZO-1 and occludin) of the gut barrier (Kozakova et al. 2016). In addition, the Nrf2 pathway was recently reported to be regulated in vivo by *L. plantarum* (Jones et al. 2015), and the host response signatures of small and colonic tissues induced by 53 gut symbionts in monocolonized mice were shown to be strain specific (Geva-Zatorsky et al. 2017).

Microbiome

Unlike elusive host genotypes, the gut microbiome provides direct evidence of host factors influencing colonization. In human FMT trials, the engraftment of fecal bacteria can be largely predicted from the abundance and phylogeny of the microbiome in the donor and the pre-FMT patient (Smillie et al. 2018). Some factors such as age (Spor et al. 2011), gender (Frese et al. 2011) (**Supplemental Table 1**), and medication (Denou et al. 2008) (**Supplemental Table 1**) may affect colonization by incoming strains partly via microbiome modification.

Supplemental Material >

From an ecological perspective, two seemingly paradoxical theories of phylogenetic limiting and phylogenetic clustering coexist. The notion of phylogenetic clustering, also known as the "like will to like" rule, has been addressed based on habitat filtering pressure, by which the host selects gut microbial consortia with common traits. The presence of closely related species in the resident microbiome can increase the chance of invasion by newly incoming species. Indeed, a microbiome with higher titers of *Lactobacillus* was shown to be more efficiently colonized with a commensal *L. reuteri* after oral administration (Stecher et al. 2010).

However, other work supports the phylogenetic limiting concept. *B. longum* AH1206 engrafts in a subset of volunteers, so-called persisters, whose gut microbiota were not rich in the species *B. longum* or genes for carbohydrate use (Maldonado-Gómez et al. 2016). Similarly, Zmora et al. (2018) found an inverse relationship between baseline levels of probiotic species and their fold change during treatment (human subjects treated with an 11-strain probiotic cocktail). Sequential

introduction of two isogenic *B. thetaiotaomicron* strains into the mouse gut caused a near absence of the later incoming strain (Lee et al. 2013). Which of these two driving forces dominates is likely to be taxon- and context-dependent and determined by the rivalry between host selection and resource competition within this niche. Also, phylogenetic clustering, characterized by the gut environment suitable for a subset of microbes, tends to occur among strains from wide phylotypes (from the same phylum or genus), and phylogenetic limiting, which is characterized by tight niche and resource competition, is more common in those from narrow phylotypes (from the same species or strain).

PREDICTION OF PERSONALIZED COLONIZATION BY ENGRAFTED STRAINS

Gut colonization is a multifactorial event, and this complexity could be embraced to predict engraftment. Given that the relationships between colonization phenotypes and the different factors (ingested bacteria, host aspects, microbiome, and diet) that can affect gut colonization phenotypes of bacteria (as summarized in **Figure 2** and **Figure 5**) are nonlinear and the predictor should support both categorical and continuous inputs, models based on optional regression algorithms such as gradient-boosting regression, random forest, and neural networks are methodologically suitable for the prediction. Although these algorithms have not been applied to the colonization aspect, some of them have been validated in the prediction of individualized metabolic responses (Ben-Yacov et al. 2015), health outcomes under various diets in large cohorts of humans (Chen et al. 2018, Forster et al. 2016), and microbiota engraftment after FMT in 19 patients with recurrent *Clostridium difficile* infection (Smillie et al. 2018). Each algorithm for such colonization prediction needs further evaluation via various clinical data sets. Here, we use the gradient-boosting regression algorithm (Friedman 2001) as an example to propose a personalized intervention concept for the prediction of colonization in the gut by orally administrated strains (**Figure 5**).

IMPLICATIONS AND CHALLENGES

We should be clear that colonization is not a strict requirement for the beneficial effects provided by probiotics. However, understanding how to engineer stable engraftment is probably a valuable feature for the treatment or prevention of chronic diseases. The summary of gut colonization mechanisms provided above (**Figure 2**) not only aids in the interpretation of current findings on colonization by bacterial species (especially *Lactobacillus* and *Bifidobacterium*) but also provides guidance for the development of novel and improved biotherapeutic agents.

Although most current commercial probiotics are allochthonous strains, the importance of bacterial natural history and coevolution suggests that we should apply autochthonous members of human gut microbiota if we have a goal of stable colonization. From a coevolutionary perspective, autochthonous strains are more likely to exert beneficial effects, whereas allochthonous strains might more strongly stimulate the immune system. Other factors, such as genetic characteristics, metabolic activity, immunogenicity, and the synergistic effects of colonists, are proposed to be understood via genomic analysis due to the accumulation of available sequenced bacterial genomes. Such in silico analysis could greatly expand and facilitate rational selection of bacteria for the purpose of gut colonization. Together with in vitro and in vivo assays, we have opportunities to screen or bioengineer a good colonizer; performing structural analysis of bacterial surface molecules will assist us to further understand and predict the molecular mechanisms of host–microbe interaction. The central role of diet provides opportunities to improve colonization via the addition of prebiotics (**Figure 4**) to give privileged nutrient access for ingested strains. The importance of

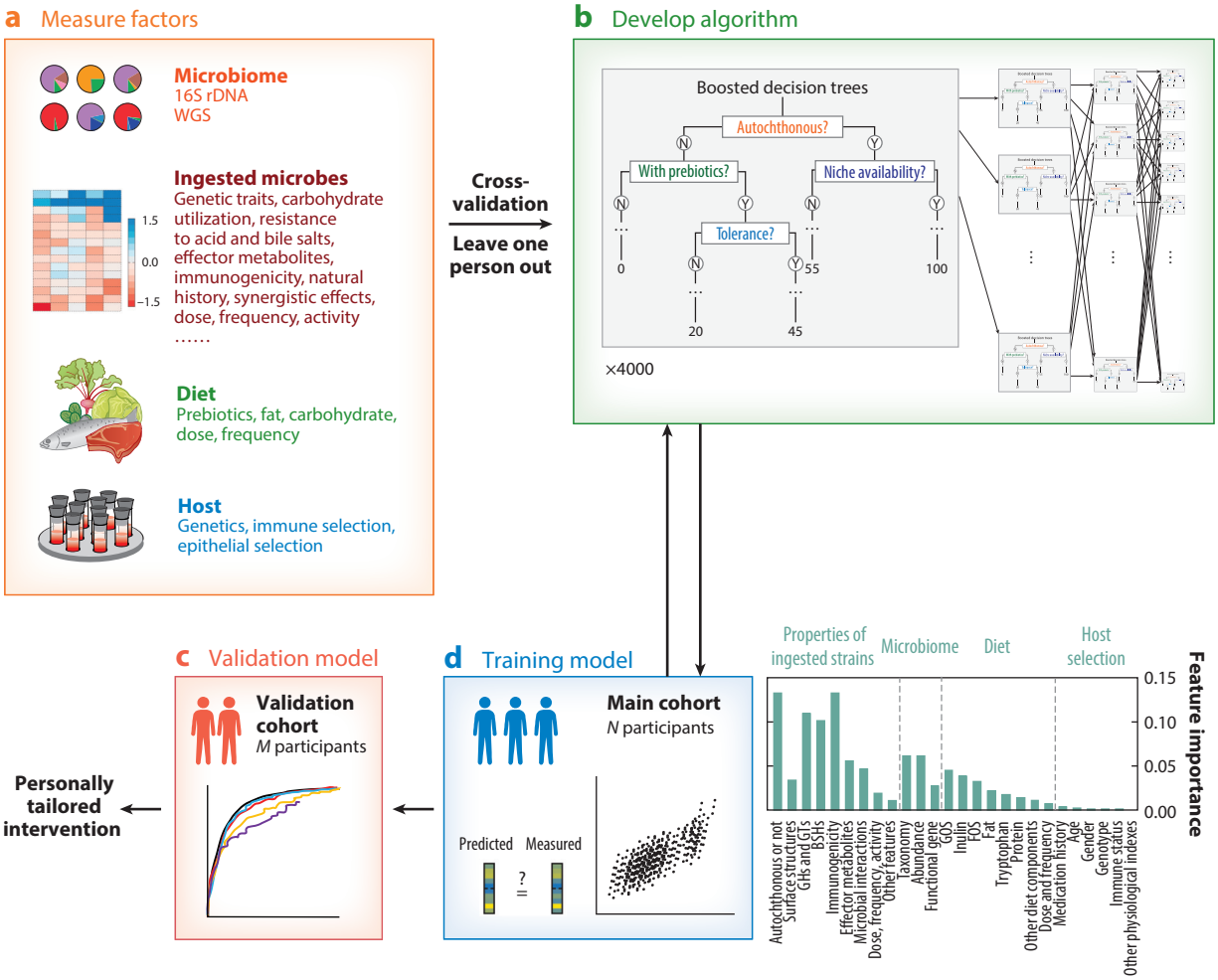

Figure 5

The concept of a personalized strategy based on a machine-learning algorithm to predict strain colonization. The ingested microbe, microbiome, diet, and host aspect factors are integrated into an algorithm that predicts individualized colonization by incoming strain. A two-stage approach is employed. In the discovery stage, the main cohort of *N* participants is used to develop the algorithm. A leave-one-out cross-validation tactic is adopted to evaluate model performance, whereby bacterial colonization in each subject is predicted using a model trained on the data of all other subjects. In the following validation stage, an independent cohort of *M* subjects is recruited, and their individualized colonization modes are predicted using the model trained only on the main cohort. The model is based on gradient-boosting regression and predicts colonization of incoming strains using the sum of thousands of different decision trees. The algorithm infers all the trees sequentially and trains each tree on the residual of all previous trees with an accumulated contribution to the overall prediction. Each tree contains the specific features that represent the properties of ingested microbes, microbiome, diet, and host aspects. To reveal factors underlying individualized prediction, the relative importance of each feature can be analyzed via partial dependence examination. Abbreviations: BSHs, bile salt hydrolases; FOS, fructooligosaccharide; GHs, glycoside hydrolases; GOS, galactooligosaccharide; GTs, glycosyltransferases; WGS, whole-genome shotgun.

genetic diversity and metabolic interactions suggests the application of designed probiotic consortia if long-term colonization is the aim. The priority effects suggest that introducing strains in early life or following antibiotic treatment can open a window of opportunity for long-term residence and may even program microbiome assemblage. Finally, no matter which of the two rules,

phylogenetic limiting or phylogenetic clustering, dominates, incoming strains should be compatible with both the phylogenic and functional repertoire of the indigenous microbiome, and it is likely that species- or strain-level competition might dominate due to the high similarity of the requirement on the niche and other resources, whereas synergistic effects are likely to occur at the genus and phylum levels.

However, screening or designing potential colonists, developing synergistic symbiotic formulations, and predicting individual-specific colonization remain easier to propose than to perform. Commonly, the mechanistic findings from mouse models are used to define the properties of potential colonists. Significant anatomical differences between the digestive tracts of rodents and humans provide caveats for this translation. Also, although an IVS model was used to design symbiotics, negative results on synergism have been reported (Krumbeck et al. 2018). In all circumstances, dose and frequency effects should be considered. The model inputs for any current colonization predictor are very prescribed, and therefore more well-controlled clinical data are needed.

Nonetheless, we are confident that we are approaching a situation in which it should soon be possible to select or engineer a strain or consortium with long-term colonization as a goal, although the interindividual variability of the host (the superorganism comprising the human and microbial biomes) represents a formidable barrier to any potential interloper.

DISCLOSURE STATEMENT

The authors are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

AUTHOR CONTRIBUTIONS

Y.X. and Q.Z. collected all the related materials, drew the figures, constructed the tables, and drafted the manuscript. H.Z., W.C., and C.H. conceived the topic and the outline. C.H. edited and polished the manuscript. All authors read and approved the final manuscript.

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