

# The Yin and Yang of Bacterial Resilience in the Human Gut Microbiota

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## Abstract

The human gut is home to trillions of microbes that form a symbiotic relationship with the human host. During health, the intestinal microbiota provides many benefits to the host and is generally resistant to colonization by new species; however, disruption of this complex community can lead to pathogen invasion, inflammation, and disease. Restoration and maintenance of a healthy gut microbiota composition requires effective therapies to reduce and prevent colonization of harmful bacteria (pathogens) while simultaneously promoting growth of beneficial bacteria (probiotics). Here we review the mechanisms by which the host modulates the gut community composition during health and disease, and we discuss prospects for antibiotic and probiotic therapy for restoration of a healthy intestinal community following disruption.

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## Introduction

The human intestinal microbiota is continually subject to a wide variety of perturbations, including host immune response, nutritional variations, and the invasion of new species. Even in the face of these affronts, the gut microbiota is generally stable over time [1] due to the resilience of commensal microbes to survive under continuous challenge. This stable microbial community consistently provides a set of services to its human host, including protecting against enteric pathogens [2], liberating nutrients from food [3], and signaling immune system regulation [4]. When the healthy gut microbiota is disturbed and one of these services breaks down, there is an urgent need to restore a healthy configuration. This is a daunting task for researchers and clinicians as the optimal composition of healthy, normal-functioning, gut microbiota is still unclear. Modulation of the gut microbiota through antibiotic therapy (to eliminate pathogenic bacteria)

and probiotic and prebiotic administration (to promote growth of beneficial bacteria) has been shown to be effective treatment strategies for restoring healthy function; however, they are not without their challenges.

Historically, antibiotics have been largely effective at eliminating enteric pathogens, but the continual rise in rates of antibiotic resistance [5], through mutation and horizontal gene transfer (HGT), has necessitated development of new treatment strategies to fight pathogenic bacteria. Complicating matters further, antibiotic treatment often perturbs the highly diverse gut community and can lead to a decrease in microbiota-mediated colonization resistance, sometimes resulting in colonization and growth of resistant pathogens [6]. The same colonization resistance encoded by the commensal microbiota that helps protect against pathogen invasion can also be a significant obstacle in effective probiotic therapy. With the continuing spread of antibiotic resistance and the growing prospects for probiotic therapy, new research has focused on the challenges that bacteria face in the human intestine—how we can increase those

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challenges for pathogens and how we can engineer probiotics to overcome those challenges in an attempt to rescue and maintain normal gut microbiota function.

### Challenges to bacterial survival and colonization of the gut environment

#### *Host modulation mechanisms of gut microbial communities*

The mammalian large intestine seems like an ideal environment for bacterial life, with a regular flow of nutrients and protection from environmental fluctuations; however, microbial residents also face many challenges for survival and growth. One of the greatest challenges that bacteria face in the large intestine is the very mechanism that brings nutrients to them: peristalsis. It is estimated that peristalsis removes tens of millions of viable bacterial cells from the vertebrate intestinal environment each day [7]. A recent genetic screen in nematodes identified several defects in peristalsis that lead to hypersusceptibility to pathogenic infection [8]. In order to successfully survive in and colonize the human gut, many bacteria have evolved machinery specialized for adhesion to human epithelial cell receptors, and these “adhesins” have been shown to be necessary for persistence of several strains in the gut microbiota [9–12]. The human host, in turn, evolved a part of the adaptive immune system to block association with the epithelium, secretory immunoglobulin A (SIgA). SIgA is secreted into the intestinal mucosa in large quantities and, like other antibodies, is produced against specific surface antigens but has also been shown to interact with some bacterial adhesins through a separate, non-specific binding domain [13,14]. For both its specific and non-specific binding mechanisms, it is believed that SIgA binds bacteria in the mucus layer and blocks them from adhering to or invading intestinal epithelial cells. Using SIgA targeted to lipopolysaccharide and the intestinal pathogen *Shigella flexneri*, Mathias *et al.* demonstrate that SIgA not only blocks proteins that mediate binding or invasion but also can cause agglutination of the target bacteria, slowing their growth rate and preventing contact with intestinal epithelial cells [15]. The bacteria bound by SIgA are then removed from the intestine by mucociliary movements [13].

The human host also deploys more deadly measures to modulate bacterial communities in the large intestine, including the oxidative stress and antimicrobial peptide (AMP) components of the innate immune system. Each of these systems has broad antimicrobial activity, making them a challenge for pathogenic and symbiotic bacteria alike. Oxidative stress in the intestinal environment is caused by a variety of reactive oxygen species (ROS) and reactive nitrogen species (RNS), including peroxide, superoxide, and nitric oxide [16,17].

These highly reactive molecules damage all forms of cellular macromolecules and show no specificity for organism type. Because of the damage it can cause to human cells, oxidative stress is generally only employed by the immune system when the body senses a serious assault. Gut epithelial cells produce ROS and RNS primarily during infrequent inflammatory responses [16–19] and this production is greatly escalated during inflammatory bowel disease (IBD) [20]. Bacteria in the human intestine must be resilient to oxidative stress if they are to survive and stably colonize the human gut.

AMPs are a broad class of molecules that include the  $\alpha$ - and  $\beta$ -defensins, RegIII $\alpha$ , and cathelicidins in humans [21]. Most known AMPs act on the bacterial outer membrane or cell wall, which leads to loss of cell integrity, but some AMPs have been shown to have additional intracellular targets, including DNA, RNA, and protein synthesis [22]. As AMPs targeting the cell membrane usually recognize conserved structural molecules, such as Lipid A, rather than proteinaceous receptors, they are generally active against broad classes of bacteria, rather than specific strains [21]. Unlike ROS and RNS, some AMPs in the large intestine, such as  $\alpha$ -defensins, are expressed constitutively, though recent microarray analysis has shown significantly higher  $\alpha$ -defensin expression in inflamed *versus* non-inflamed intestinal tissue [23]. Other AMPs are activated by the presence of specific bacterial strains. For instance, recent work in gnotobiotic mice and in human tissue culture has shown that mouse RegIII $\gamma$  and its human homolog RegIII $\alpha$  are induced in response to introduction of *Bifidobacterium breve*, but not a commensal *Escherichia coli* strain [24]. Regardless of the trigger for AMP expression, their broad activity ensures that much of the microbiota will be affected whenever AMPs are deployed.

#### *Microbe–microbe competition and niche specificity*

In addition to challenges imposed by the human host, intestinal microbes also face competition from each other in order to survive. The primary driver of competition between microbes is niche specificity and nutrient availability. The niche specificity of the majority of microbes remains unclear; however, nutrient requirements have been identified for a few key gut microbiota residents [25–28]. A study of oligosaccharide usage found that *Bifidobacterium infantis*, a common member of the infant microbiota, relies upon oligosaccharides from milk, while *Bacteroides thetaiotaomicron*, common in infant and adult microbiotas, can utilize oligosaccharides from both milk and the mucus layer of the large intestine [29]. This highlights the importance of carbon metabolism in long-term survival in the microbiota. Furthermore, it has been demonstrated that niches within the gut environment are saturable and can only support a

finite population [30], resulting in an unequal competition for niches that favors established strains over invading strains. This dynamic was made evident by Lee *et al.*, who showed that a gnotobiotic mouse mono-colonized with *Bacteroides fragilis* will block colonization by a second inoculation of the same strain. They also reported that this effect is generalizable to three additional *Bacteroides* strains [30].

Strains in an established microbiota can employ several strategies to outcompete invading strains. One is physical exclusion: *B. thetaiotaomicron* has been found to attach to food sources in the intestinal environment of a gnotobiotic mouse [31], which would give *B. thetaiotaomicron* an advantage in accessing essential nutrients over free floating bacteria. Other work has shown that members of the microbiota can deploy their own antimicrobials to gain a competitive advantage [32]. Some pathogens have evolved strategies to circumvent direct competition, such as *Salmonella typhimurium*, using hydrogen generated by the microbiota as an electron source in early stages of infection [33].

The niches available to the microbiota and invading strains will differ between individuals and within an individual over time, depending upon a wide variety of factors such as diet [34] and host mucus composition. Recently, McNulty *et al.* utilized a model community in gnotobiotic mice to examine fitness changes in response to dietary changes, focusing on *Bacteroides cellulosilyticus* WH2. They found WH2 to be very fit in both high-fat and low-fat diets, while the other strains in the model community showed more variable fitness [34]. In addition, host mucus composition, driven by host genetics, was shown to affect microbiota composition by Kashyap *et al.* using gnotobiotic mice with and without a functional fucosyltransferase gene [35].

#### *Anthropogenic antibiotics*

In addition to challenges imposed by the human host immune system and cohabitating microbes, the human gut microbiota is often exposed to high levels of anthropogenic antibiotics (i.e., antibiotic use in the clinic and in agriculture). Antibiotic therapy can result in drastic changes to the gut microbiota composition, with some changes persisting for years following treatment [36–39]. These changes are often asymmetric, as some bacteria have higher susceptibility than others to any particular antibiotic treatment. However, the popularity of broad-spectrum antibiotics, such as the  $\beta$ -lactams, means that the majority of human gut microbes face challenges imposed by common antibiotic therapy. Even among the survivors, fitness can be variable—some species increase fitness without competitors, while others cannot survive with the loss of other bacterial strains that they depend on [38,39].

#### **Reducing gut colonization and growth of harmful bacteria**

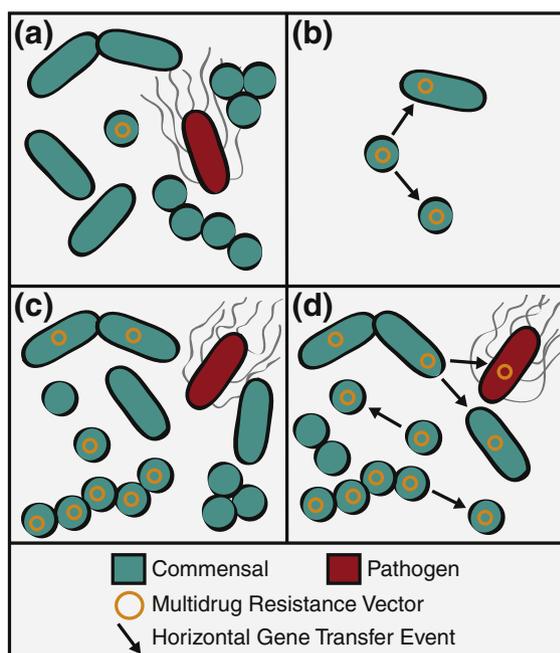
##### *Pathogen resilience mechanisms to host immune response*

In spite of the apparent challenges to growth and survival in the human intestine, enteric pathogens are still able to invade and, in some cases, even thrive. The various components of the human immune system have evolved to identify and eliminate pathogenic bacteria while allowing symbiotic species to remain. In turn, pathogenic bacteria have had the opportunity to evolve complex systems to defend against attacks by the human immune system or even to subvert them for their own gain [19]. For instance, *Vibrio cholerae* strains have been shown to avoid SIgA by down-regulating certain receptors *in vivo* [40], while pathogenic *E. coli* strains can express a specific SIgA binding antigen that interferes with immune signaling [41]. Resistance to AMPs has also been observed primarily through avoidance by modifying target molecules [42,43], while oxidative stress resistance takes the form of detoxifying enzymes such as catalase, peroxidase, and superoxide dismutase [44,45]. One way that researchers have sought to complement the immune system and overcome pathogen resilience is through the use of AMPs not naturally produced by the human host [21,22]. These antibiotics would have the advantages of AMPs, such as low levels of resistance and structural similarity to natural products of human cells, but could be administered similar to other anthropogenic antibiotics. Unfortunately, the first AMP antibiotics to be used, the polymyxins [46], also block eukaryotic translation and thus show toxicity to human cells [47]. Continued work in this field may yet yield new, safe, antimicrobial compounds, but for now, clinicians must work with more traditional antibiotic classes.

##### *Antibiotic treatment strategies for combating increasing pathogenic resistance*

The rapid evolution and expansion of antibiotic resistance in pathogenic bacteria has made treating infectious disease, while allowing growth of healthy commensal microbes, particularly challenging. Pathogenic bacteria have proven to be exceptionally resilient, continually evolving resistance to every antibiotic that has been deployed against them within a short period after introduction [5]. For a brief period, the rise in antibiotic resistance was matched by the development of new antibiotics, but in recent decades, antibiotic development has not kept pace [48], resulting in the need for new interventions for treatment of infectious disease.

Three major strategies have been proposed to keep our current arsenal of antibiotics relevant: synthetic tailoring of antibiotic side groups [49,50], antibiotic



**Fig. 1.** Antibiotic therapy increases prevalence of antibiotic resistance available to pathogens harbored in the commensal microbiota. (a) Human intestinal microbiota is invaded by a pathogen. (b) With antibiotic treatment, the pathogen is eliminated along with much of the commensal community. Several strains survive by acquiring a multidrug resistance plasmid from another community member. (c) Pathogen invades recovered community. (d) During subsequent antibiotic treatment, the pathogen has an increased likelihood of acquiring the multidrug resistance plasmid.

combinations [51–53], and antibiotic cycling [54,55]. Each of these strategies is applied to eliminate pathogens, but they often have high collateral damage, disturbing the entire gut microbiota community. Synthetic tailoring is the modification of side groups in an antibiotic molecule to extend its effectiveness or circumvent antibiotic resistance, while maintaining the core antibiotic mechanism [49]. For example, the  $\beta$ -lactams are a class of antibiotics that have undergone several levels of modification since penicillin was first discovered. The effectiveness of penicillin is confined to specific Gram-negative bacteria, but synthetic tailoring generated new antibiotics such as piperacillin and methicillin that have expanded activity [50]. The major limitation of synthetic tailoring is that it does not change the fundamental mechanism of the antibiotic. While synthetic tailoring can bypass some types of antibiotic resistance, for others, a single antibiotic resistance gene can give resistance to an entire class of antibiotics, and the latter form of resistance gene is increasing in prevalence. For instance, the recently discovered, plasmid-born *kpc* and *ndm* genes encode for enzymes that can degrade all types of  $\beta$ -lactams, and prevalence of these genes continues to increase in hospitals worldwide [56,57].

Antibiotic combinations have been used to treat organisms for which a single antibiotic treatment is insufficient, and successful combinations often exhibit synergy between the constituents. In a synergistic interaction, the effectiveness of the combination of drugs at a given concentration is greater than the effectiveness of either antibiotic on its own at that concentration. The major advantage of synergistic combinations is that they lower the total drug concentrations needed for killing, which can reduce the toxicity of the treatment to human cells [51,53]. Unfortunately, while lower total concentrations are an advantage in terms of toxicity, they are a disadvantage in terms of evolution of resistance, as bacteria evolve resistance more quickly to synergistic drug combinations than to the drugs used singly [52]. This occurs because individual drugs are dosed at sub-therapeutic concentrations, resulting in bacteria facing a lower evolutionary barrier to become resistant to each component of a combination. Once a bacterium evolves resistance to one component, the synergy is broken and other components are no longer at killing concentrations, overall increasing rates of evolution of resistance. This downside has been explicitly demonstrated for two-drug combinations [52], but the theoretical principle may also apply for higher-order synergistic compound combinations.

While antibiotic cycling is an established concept [58–60], the idea has received renewed recent interest in the context of the phenomenon of collateral antibiotic sensitivity. An antibiotic is considered to confer collateral sensitivity if resistance evolved to that antibiotic makes a bacterium more susceptible to another antibiotic, compared to the wild-type population [55]. In some cases, two antibiotics can be reciprocally collateral sensitive, where resistance evolved to either antibiotic increases susceptibility to the other. In this case, it has been proposed that one antibiotic could be applied until resistance to that antibiotic is manifested and then treatment switched to the other [54]. This cycling process could be repeated until the infection is cleared, and since exposure to each antibiotic selects for susceptibility to the other, there would be no net evolution of resistance. This procedure holds much promise, but cycling based on reciprocal collateral sensitivity has yet to be implemented clinically, and it is not known how generalizable collateral sensitivities are between species or even strains.

#### *The commensal resistome as a resilience factor*

Each of the antibiotic treatment strategies outlined above provides benefits for treating pathogens individually, but they do not adequately address the additional resilience factors available for transfer to pathogens with access to the commensal microbiome.

HGT between bacteria has made a major contribution to the increase in antibiotic resistance, as evidenced by the worldwide spread of specific  $\beta$ -lactamases [48]. The various mechanisms of HGT have been extensively reviewed elsewhere [61,62], but one of their cumulative effects has been to greatly expand the diversity of genetic material available to pathogens, well beyond what is found in any single genome. The effects of HGT on clinical outcomes can be seen in studies of the spread of particular resistance genes, such as the CTX-M  $\beta$ -lactamases [63]. The complete set of antibiotic resistance genes present in a microbial community is known as the “resistome” and previous research has shown that the resistome of both human adult and pediatric intestinal microbiota is far more diverse than what has been seen in pathogens [64,65]. It has also been shown that the collection of resistance genes accessible to pathogens extends well beyond the human microbiota, including animal [66] and soil [67] environments. Antibiotic therapy, therefore, does not only select for increased antibiotic resistance in pathogens but also increased prevalence of antibiotic resistance genes in microbial communities available for transfer to pathogens (Fig. 1).

Resistance acquired through HGT poses an immense challenge to clinical treatment of pathogens because it decouples phylogeny from antibiotic resistance profile. Tests for taxonomic identity of a pathogen can return results 1–2 days faster than tests for antibiotic resistance in rapidly growing pathogens. For vertically inherited resistance mechanisms, taxonomic identity can provide insight into the susceptibility and treatment options; however, horizontally transferred resistance genes can distort this inference. Our current antibiotic treatment strategies are not adequate to combat pathogens with access to the commensal resistome through HGT. Synthetic tailoring and combination therapy provide for selection of increased antibiotic resistance in the entire microbiota and, thus, increase the size and diversity of resistomes. Antibiotic cycling with reciprocally collaterally sensitive antibiotics shows promise in slowing evolution by mutation, but it remains to be seen whether collateral sensitivity cycling is robust to resistance acquired by HGT. Continued research into new treatments, especially treatments that can eliminate pathogens without increasing the resistome, therefore remains a pressing need.

### Promoting growth and colonization by beneficial bacteria

#### *Colonization resistance for beneficial bacteria*

As the fight against multidrug resistance in human pathogens continues to escalate, there is simultaneous interest in promoting the growth and stable colonization in the human gut by beneficial bacteria (probiotics) that confer health benefits to the human host. Probiotics have shown promise in treatment

of a variety of diseases, including IBD [68], atopic disease [69,70], lactose intolerance [71], pathogen-associated diarrhea [72], and necrotizing enterocolitis [73,74]. A major component of the potential of probiotic treatment for chronic diseases is the possibility for stable integration of the probiotic into the microbiota. In this case, colonization of the microbiota could provide long-term benefits against chronic conditions such as IBD without the need for continual administration. However, while probiotics have been effective at treating gastrointestinal disorders such as acute infectious diarrhea and necrotizing enterocolitis in infants and young children [75,76], limited long-term positive results have been reported from clinical trials using probiotics in adults, possibly due to the inability of probiotics to survive and colonize the existing gut community. For example, *E. coli* Nissle 1917 and *Lactobacillus rhamnosus* GG (LGG) are two clinically relevant probiotic strains that have been shown to be effective in treatment of gastrointestinal disease [75,77,78]. However, after administration is terminated, both probiotics are cleared from the majority of patients within weeks. In a study of 48 healthy adult individuals who were administered *E. coli* Nissle for a run-in period of 17 days, only approximately 45% of the individuals had detectable levels of the probiotic 2 weeks after stopping administration [79]. This number continued to drop continuously until the probiotic was undetectable in nearly all individuals after 48 weeks. Similarly, in a randomized trial of 36 individuals who consumed LGG for a 2-week run-in period, administered as capsule, yogurt, or cheese, only about 30% of individuals had detectable levels of LGG after a 3-week period, regardless of route of administration [80]. In the same study, other probiotic strains of *L. rhamnosus* were undetectable in the gut microbiota of 100% of individuals after 3 weeks. These results highlight the importance of understanding and improving resiliency functions and colonization of probiotic strains in the human gut microbiota.

Resistance of the stable adult gut microbiota to colonization by exogenous probiotic species may be the result of established bacterial strains filling all the available metabolic and physical niches; thus, newly introduced organisms must compete against them for colonization, growth, and expansion. This type of “colonization resistance” mediated by the composition of resident gut microbiota has long been recognized as a defense mechanism against pathogenic bacteria [6,81,82]. Disruption of the commensal microbiota by antibiotic therapy reduces colonization resistance through reduction in the commensal microbial abundance and species diversity, thereby freeing niches and nutrients for exogenous microbes to exploit. Post-antibiotic enteric pathogen expansion is especially apparent in *Clostridium difficile* infections, where exposure to broad-spectrum antibiotics is the strongest predictor of *C. difficile* expansion in humans [83] likely due to niche clearance of commensal microbes.

One study demonstrated that the increase in sialic acid and fucose following the relative reduction in commensal microbes by antibiotic therapy enabled the expansion of two enteric pathogens, *C. difficile* and *S. typhimurium* [84]. *C. difficile* capitalized upon the free sialic acid, while *S. typhimurium* utilized both host sugars originally liberated by commensal bacteria.

#### *Functional mechanisms of gut microbiota colonization by commensals*

Functional mechanisms promoting colonization of the human gut have been extensively studied in a handful of important gut microbial inhabitants. Nutrient availability and utilization has been repeatedly identified as an important contributor to gut colonization by various bacterial strains. For example, gene clusters conserved among intestinal *Bacteroides* species, termed polysaccharide utilization loci (PULs), have been long known to control specificity of polysaccharide use and have been shown to play important roles in bacterial persistence and colonization in the gut [85]. As a result, *Bacteroides* persistence and colonization in the gut is highly dependent on host diet and mucosal polysaccharide composition [86]. For instance, the species-specific bacterial exclusion demonstrated by Lee *et al.* (described above) is mediated by a unique class of PULs. In addition, the authors showed that these PULs are necessary for *Bacteroides* colonization following disruption of the microbiota [30]. Nutrient utilization and specificity also plays important roles in gut colonization by pathogenic bacteria during inflammation. Deriu *et al.* demonstrated that administration of probiotic bacteria *E. coli* Nissle 1917 reduced colonization by *Salmonella enterica* Typhimurium through competition for limited available iron in the host gut environment [87].

In addition to nutrient utilization, direct suppression of the host immune response by commensal bacteria can allow niche-specific colonization of commensals on mucosal surfaces while retaining defenses against bacterial pathogens. A recent study identified a unique surface polysaccharide of *B. fragilis* that binds toll-like receptor 2 on CD4+ T cells, which induces IL-10 production [11]. This, in turn, leads to suppression of Th17 cell responses and allows colonization of *B. fragilis* in the gut epithelium. This has been proposed as a mechanism by which the human host discriminates between commensal and pathogenic bacteria and a mechanism by which beneficial bacteria evade the challenges imposed by the host for colonization and survival in the human gut.

#### *Promoting growth of beneficial bacteria through nutrient modulation*

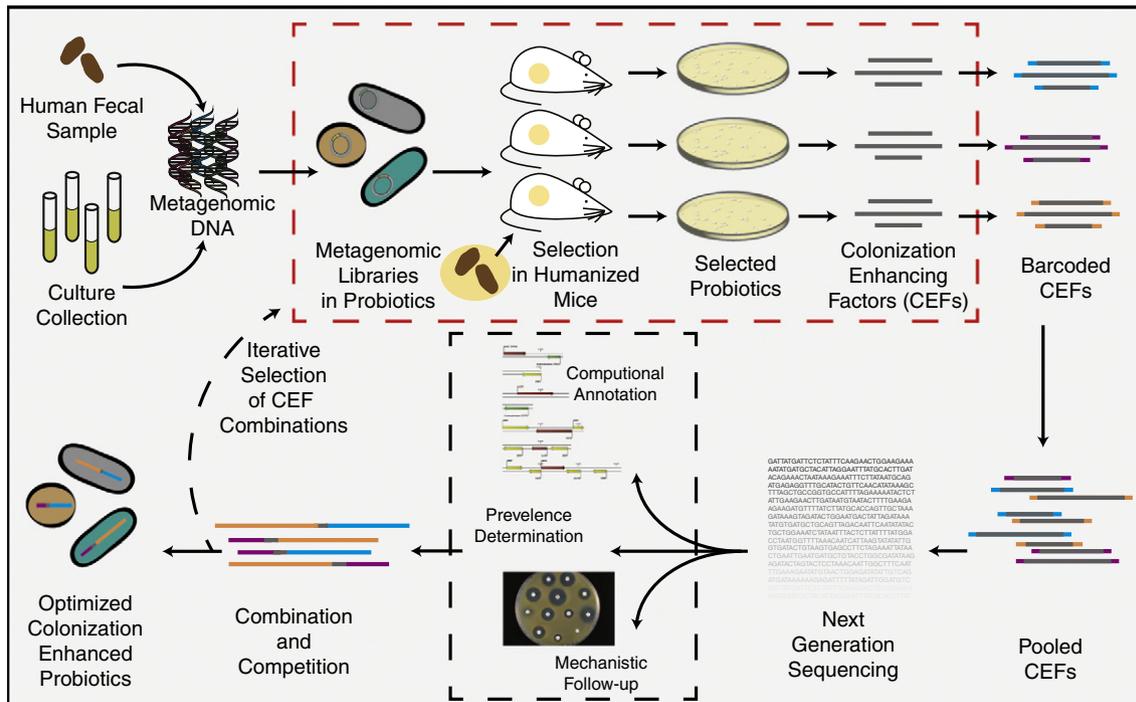
Promotion of growth of beneficial bacteria can also be achieved through the administration of prebiotics or

nondigestible carbohydrates that can alter the composition of the gut microbiota community by selectively enriching for beneficial bacteria. Oligofructose and inulin are two of the most studied and well-established prebiotics, promoting growth of Bifidobacteria and Lactobacilli, which have specialized in utilization of a wide variety of carbohydrates [88,89]. In theory, prebiotics and probiotics can be administered together in order to promote colonization and growth of the probiotic. This combination therapy was coined synbiotics, referring to the synergistic beneficial effects of both probiotics (beneficial bacteria) and prebiotics (their associated nutrient needs) over either component alone. Synbiotics have shown promising outcomes in treatment of intestinal dysbiosis [90] and recovery from infection by enteric pathogens [91].

#### *Genetic engineering of probiotics for improved bacterial resilience and colonization*

For the full utility of probiotic therapy to be realized for chronic conditions, stable colonization of existing gut microbiota by exogenous microbes is necessary. Genetic engineering of existing probiotic bacteria to improve colonization potential holds great promise but is severely hindered by the lack of an understanding of universal and *transferable* mechanisms of bacterial colonization and fitness in the gut microbiota, such as tolerance to bile salts [92] or degradation enzymes present in the intestines (e.g., lysozyme [93]). Bioprospecting microbial communities using functional metagenomic selections in the host strain of interest provides a powerful technique for identifying transferable resilience mechanisms and colonization factors for commensal bacteria and has been previously utilized as a method for generating a synthetic toolbox for microbial engineering [94]. Functional screening of the murine gut microbiota has previously been used to successfully identify multiple colonization factors that function in *E. coli* [95]. The identified genetic determinants are likely of *Bacteroides* origin; however, the mechanism by which they enhance intestinal colonization remains unknown.

Genetic engineering for enhanced probiotic species still has long ways to go before it can be applied clinically; however, *in vivo* bioprospecting of colonizing human gut microbiota in known probiotics can be employed in order to speed up the pipeline from engineering to clinical application (Fig. 2). In addition to resilience functions for survival in the harsh human gut environment, the species composition and niche availability of the resident gut microbiota is an important consideration in enhancing colonization. Given that universal colonization factors may not exist, functional selection of colonization factors in gnotobiotic mice colonized with intact gut microbial communities from human donors ("humanized" mice) holds promise for genetic engineering of personalized probiotics



**Fig. 2.** Genetic engineering of probiotics for enhanced colonization of gut microbiota. Bioprospecting of actively colonizing and established human gut microbiota, and culture collections including commensal bacteria, can allow identification of transferrable colonization enhancing factors. Here we show generation of metagenomic libraries in existing probiotics and selection against a humanized gut microbiota murine model. The identified colonization enhancing factors can then be barcoded, sequenced, and assembled using methods developed for functional metagenomic selections of antibiotic resistance. Mechanistic follow-up of identified colonization enhancing factors is essential as an understanding of universal colonization factors is currently lacking. Engineering of synthetic operons and iterative selections can enable generation of optimized probiotics capable of stable colonization of the human gut microbiota.

for enhanced colonization based on the existing gut microbiota community composition specific to an individual. An intermediate step in this ultimate engineering goal would be identification of colonization and fitness factors that improve synergy between existing probiotics and prebiotics. Functional selections for colonization and fitness factors in the presence of various prebiotics and in response to chow representative of human diets worldwide have the potential to identify transferable metabolic mechanisms that utilize specific nutrients that are can be supplemented and are naturally available in the gut from the foods we eat. As we learn more about the niche specificity of gut microbe residents and are able to predict available niches, we may draw from the functional toolbox selected in the presence of prebiotics in order to engineer probiotics and promote colonization on a personal basis based on current gut microbiota composition and/or host diet.

While genetic engineering of probiotics holds great promise in the era of personalized medicine, this prospect still faces significant challenges before translation to a clinical setting. Permanent colonization of the gut microbiota is desirable for treatment of chronic diseases; however, as is the focus of this

review, there are always trade-offs to increased resiliency and it is necessary to ensure that engineered probiotics do not bloom and take over the current microbial community. In addition, as discussed before, host diet is an important contributor to the fitness of bacterial inhabitants of the gut based on metabolic niches, and therefore, functional selection based on diet and nutritional supplementation may be necessary to realize the full utility of this approach.

## Conclusions and Future Perspectives

The gut microbiota is a complex ecosystem, which during health provides many essential functions to the host, including carbohydrate metabolism, modulation of the immune system, and protection against pathogen invasion. The host is intimately involved in the maintenance of a healthy gut microbial community. Resident bacteria, both harmful and beneficial, must therefore avoid and adapt to potentially lethal host immune responses in order to thrive in this environment. Disruption of this community leads to heightened immune response in the host and has been associated with a number of intestinal diseases,

including IBD such as Crohn's disease and ulcerative colitis. In addition, while the immune response has evolved to identify and eliminate enteric pathogens, colonization and outgrowth by these organisms often requires clinical intervention. The administration of antibiotic therapy for treatment of IBD and pathogenic infections is often a successful treatment option; however, it has the potential for high collateral damage, including enrichment of antibiotic resistance in human pathogens and the commensal microbiota and reduction in species composition and diversity. As antibiotic options are continuing to dwindle due to the widespread dissemination of antibiotic resistance genes, identification of alternative treatment options is necessary.

Probiotic and commensal bacteria show promise at eliminating and preventing colonization of pathogens from the gut environment; however, successful stable introduction of probiotic species into an existing gut community has met with enormous challenges. Genetic engineering of probiotics to increase colonization potential is one potential solution, not only in supplementing healthy gut microbiota function but also to outcompete pathogens for the available niches. As the functional mechanisms of transferrable colonization necessary for genetic engineering are still poorly understood, unbiased bioprospecting of commensal gut microbiota holds great promise for identification of these genetic, engineerable, building blocks.

While the exact species and phylogenetic composition of what constitutes a healthy gut microbiota is still unclear, there is a continued need to eliminate harmful bacteria (pathogens) while promoting the growth of beneficial bacteria (probiotics). A clearer understanding of resilience mechanisms of both harmful and beneficial bacterial inhabitants of the human gut microbiota should enable the design of next-generation treatment strategies for rescuing and maintaining the health of this critical microbial ecosystem and, in turn, its human host.

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### Abbreviations used:

HGT, horizontal gene transfer; SlgA, secretory immunoglobulin A; AMP, antimicrobial peptide; ROS, reactive oxygen species; RNS, reactive nitrogen species; IBD, inflammatory bowel disease; PUL, polysaccharide utilization locus.

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