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REVIEW ARTICLE

Probiotics in Action: Enhancing Immunity and Combatting Diseases for Optimal Health

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Abstract

The human microbiome, comprising a diverse array of microorganisms, plays a pivotal role in safeguarding against pathogenic threats. Probiotics have emerged as formidable allies in countering infections caused by fungi and waterborne pathogens. This review offers an in-depth examination of the mechanisms underlying the microbiome's defense against viral infections, with a specific focus on probiotic interventions. Mycotoxins, secondary compounds produced by microfungi, pose significant health risks. Yet, certain strains of Lactic Acid Bacteria (LAB) have exhibited remarkable efficacy in eliminating aflatoxin B1 (AFB1), the most toxic member of the aflatoxin family. Experimental setups demonstrated AFB1 binding to specific LAB strains, persisting even after gastric digestion. Laboratory studies revealed a potential protective mechanism wherein pre-incubation of probiotics with mycotoxins reduced their adhesion to mucus. Animal trials further underscored the benefits of oral probiotic administration, showcasing increased fecal excretion of mycotoxins and mitigation of associated health risks. Cyanobacteria-generated microcystins in drinking water pose a significant threat to human health. Probiotic bacteria, particularly strains like *Bifidobacterium longum* and *Lactobacillus rhamnosus*, have demonstrated exceptional efficacy in removing the cyanobacterial peptide toxin microcystin-LR. Optimized conditions resulted in rapid toxin elimination, highlighting the potential of probiotics in water purification. Engineered probiotics represent a cutting-edge approach to tailor microorganisms for specific therapeutic applications, exhibiting promise in treating metabolic disorders, Alzheimer's disease, and type 1 diabetes. Additionally, they serve as innovative diagnostic tools, capable of detecting pathogens and inflammation markers within the body. In the realm of antimicrobial peptide production, probiotics offer a promising platform, with genetically modified strains engineered to produce human β-defensin 2 (HBD2) for treating Crohn's disease, showcasing their potential in targeted therapeutic delivery. Biocontainment strategies have been implemented to prevent unintended environmental impacts.

Keywords: Antimicrobial Peptides, Fungal Infections, Mycotoxins, Probiotics, Waterborne Pathogens

1. Introduction

The body's gastrointestinal system is home to a wide variety of microorganisms referred to as gut microbiota. While a multitude of intrinsic factors, such as the secretion of mucin, have the potential to impact the equilibrium of these microbes, it is human nutrition that predominantly serves as the primary sustenance for their cultivation. Especially noteworthy are non-digestible carbohydrates, which wield substantial influence over the composition and operation of gut bacteria. Valuable microorganisms within the gut possess the ability to break down these non-digestible dietary components, known as prebiotics, deriving their survival prowess from the breakdown of these resistant prebiotic linkages. Consequently, prebiotics can selectively shape the dynamics of gut microbiota[1].

The term "probiotics" made its inaugural appearance in 1974, and subsequently, the Food and Agriculture Organization of the United Nations along with the World Health Organization delineated probiotics as "living microorganisms which when administered in sufficient quantities, confer a health benefit on the host"[2]. Among the probiotics commonly employed are *Escherichia coli*, *Saccharomyces boulardii*, as well as species encompassed within the *Lactobacillus* and *Bifidobacterium* genera, which encompass *Bifidobacterium infantis, Lactobacillus rhamnosus,*

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Bifidobacterium breve, Lactobacillus reuteri and *Lactobacillus casei*. Both prebiotics and probiotics yield advantageous outcomes for human nutrition and well-being[3].

In contemporary times, there has been a notable reduction in the cost of genome sequencing, accompanied by the escalating potency of tools intended for the manipulation and refinement of microbial genomes. This enhanced capability empowers us to meticulously fashion probiotics in accordance with our individual conceptions, thereby facilitating the creation of bespoke probiotic formulations[4]. Engineered probiotics stand as the imminent evolution of dynamic biotherapeutics, having undergone genetic modifications for the precise targeting of particular ailments. Fueled by the progress in the field of synthetic biology, the art of probiotic engineering has attained an elevated level of intricacy, resulting in the development of strategies for therapeutic interventions geared towards the treatment of cancer, infections, metabolic irregularities, and inflammatory conditions, while concurrently assuming roles in their detection and prophylaxis[5].

Engineering probiotics with additional enzymes or pathways that provide needed intermediates or eliminate harmful substances is a potential method for treating metabolic illnesses caused by enzyme shortage[6,7]. Modifying probiotics through engineering techniques can serve as a preventive measure against infections induced by pathogens. This involves the release of antimicrobial peptides or bacteriocins, facilitating the accelerated growth of probiotic strains[8].

Harnessing genetic manipulation to modify probiotic strains for the purpose of targeting specific diseases holds considerable promise, as it permits the imbuing of probiotics with a multitude of advantageous attributes tailored to the targeted ailment. This could potentially yield heightened efficacy in comparison to their unaltered counterparts. Therapeutic interventions centered around engineered probiotics carry a range of benefits over existing treatments, including cost-effective formulations with diminished potential for adverse effects[9,10]. In this comprehensive analysis, we have not only delved into the foundational aspects of probiotics but have also expounded upon the symbiotic relationship with a well-established prebiotic. Given the evolving ease of genetically engineering probiotics, courtesy of advancements in the realm of synthetic biology, our discussion also provides a glimpse into the operational mechanics of an engineered probiotic, supported by illustrative instances.

2. Materials and Methods

The search strategy employed for this study encompassed several databases, including PubMed, Scopus, ScienceDirect, Web of Science, and Google Scholar. Key search terms such as Probiotics, probiotic engineering, Fungal Infections, Waterborne Pathogens, Mycotoxins, and Antimicrobial Peptides and variations of these terms were utilized to identify pertinent literature. The primary focus of this research is the exploration of immune system modulation through probiotics and its associated benefits for addressing immune and other health-related issues. Exclusion criteria were applied to filter out studies not directly relevant to these topics. Additionally, reference lists of relevant articles were manually reviewed to supplement the database search, and expert opinions were sought to ensure comprehensive coverage of the subject matter.

3. Mechanism of Promoting Probiotics by Prebiotics

3.1 Prebiotics

In 2016, the International Scientific Association for probiotics and Prebiotics undertook a redefinition of prebiotics, characterizing them as substances that the host intestinal flora can selectively utilize and transform, under the condition that they have a beneficial impact on the well-being of the host. This revised prebiotic definition encompasses substances beyond carbohydrates and expands the domain of their activity beyond the gastrointestinal tract, extending to sources beyond conventional dietary intake[11]. Among the array of prebiotics, the category comprises not solely carbohydrates but also encompasses diverse non-carbohydrate entities satisfying prebiotic criteria, including polyphenols sourced from fruits like black raspberries and blueberries. While probiotics stand as a boon to our well-being, these prebiotics function as a boon to the beneficial bacteria. Functioning as specialized plant fibers, prebiotics serve as nourishing agents for probiotics. Together, the tandem of

prebiotics and probiotics provides essential support to our physiological systems. In particular, prebiotics play a pivotal role in cultivating and upholding a thriving community of beneficial bacteria, thereby fortifying the intestinal environment and facilitating the digestive processes[12]. The lack of enzymes capable of hydrolyzing the polymer bonds within prebiotics leads to their resilience against digestion within the small intestine, causing them to persist in the gastrointestinal tract. Consequently, the intact prebiotic compounds are transported by the human body to the large intestine. Within this locale, they undergo degradation mediated by the intestinal flora, undergoing selective fermentation that yields specific secondary metabolites. These metabolites are subsequently taken up by the intestinal epithelium or conveyed to the liver via the portal vein. The impact of these metabolites on host physiological processes is salutary, encompassing regulatory effects on immunity, resistance to pathogens, enhancement of the intestinal barrier's functionality, heightened mineral absorption, and reduction of blood lipid levels[13,14]. On the other hand, Probiotics elicit advantageous effects within the body through a quartet of primary mechanisms: thwarting the proliferation of potential pathogens and suppressing their growth, enhancing the gut's barrier functionality, modulating the body's immune response, and generating neurotransmitters that can influence the host's modulation[15] as summarized in Table 1.

3.2 Prebiotic: Fostering the Proliferation and Expansion of Probiotic Microorganisms

Earlier research endeavors have highlighted the capacity of prebiotics to stimulate the expansion of probiotic populations within the human gastrointestinal milieu, consequently fostering improvements in the overall diversity of intestinal microbial communities[16]. Notably, the proliferation and metabolic functions of probiotics are contingent upon the availability of carbon sources, predominantly originating from carbohydrates. Unlike prebiotics, which evade decomposition by human digestive enzymes, undigestible prebiotics can be enzymatically converted into essential carbon reservoirs within the intestinal environment. This enzymatic conversion augments the growth and multiplication of beneficial bacterial strains and contributes to the modulation of probiotic composition. A case in point is a study that demonstrated the polysaccharide component gleaned from the brown seaweed *Silvetia compressa*, which was capable of inducing the proliferation of *Bifidobacterium* and *Lactobacillus* populations. Consequently, this led to an escalation in the creation or production of total Short-Chain Fatty Acids (SCFAs), yielding effects akin to those achieved by utilizing inulin[17]. Furthermore, the impact of *Enteromorpha clathrata* polysaccharide on the microbiota exhibited differential effects in male and female subjects. In male mice, this polysaccharide led to an augmentation in the population of *Lactobacillus* bacteria. Conversely, in female subjects, it triggered an upsurge in *Bifidobacterium* and *Akkermansia muciniphila* (recognized as the succeeding iteration of probiotics)[18].

Beyond the realm of polysaccharide prebiotics, polyphenolic compounds possess the ability to discerningly foster the proliferation of beneficial bacteria. Within mangos, certain existing phenolic compounds like catechin, protocatechuic acid, gallic acid, and vanillic acid not only inhibit the growth of harmful bacteria but also exert positive effects on the expansion and reproduction of probiotics[19].

3.3 Prebiotic Enhancing the Metabolism of Probiotics

The gut microbiota engages with the human organism, facilitating the digestion and assimilation of nutrients from food, detoxifying waste generated within the intestinal tract, and synthesizing essential life-supporting compounds such as amino acids, vitamins, SCFAs, and other substances[20]. SCFAs are the resultant metabolic byproducts from the breakdown of carbohydrates and proteins through fermentation by intestinal microorganisms, arising from either internal or dietary sources. These SCFAs comprise saturated fatty acids featuring carbon chains spanning 2 to 6 atoms in length. Within the intestines, prebiotics fosters the generation of beneficial metabolites, primarily SCFAs, in the presence of probiotics, thereby shaping the intestinal milieu and reducing intestinal pH levels[21]. The number of prebiotics also has a crucial impact on influencing the quantity of SCFAs generated by probiotics. A research study conducted an investigation into five distinct prebiotics with varying concentrations, namely inulin, alpha-GOS, beta-glucan,

beta-GOS, and xylo-oligosaccharides, to examine their regulatory impact on the intestinal microbial community. The experimental findings revealed that beta-glucan exerted the most pronounced influence on both microbial composition and metabolism. As the concentrations of beta-glucan and inulin increased, there was a concurrent elevation in the proportion of butyrate, a compound known for its advantageous attributes including anti-colon cancer and anti-inflammatory properties[22]. Generally, prebiotics stimulate the synthesis of SCFAs, predominantly acetic acid, butyric acid, and propionic acid, by probiotics. The escalation in SCFA levels also results in a reduction in intestinal pH, contributing positively to human well-being[23].

3.4 Strengthening Probiotic Resistance to Reactive Oxygen Species (ROS) and Bile Salts/Acids through Prebiotics

Research indicates that plant inulin, fructans, polyphenols, and yellow lupin polysaccharides, known as prebiotics, possess the ability to neutralize free ROS within the gastrointestinal tract, affording protection to probiotics. This protective mechanism arises from the production of butyric acid as part of SCFAs metabolism, which consumes oxygen during its gut processing[24]. Notably, most intestinal probiotics are highly vulnerable to bile salts/acids, as these compounds can hinder probiotic growth and functionality of probiotics by triggering oxidative stress, breaking down bacterial membranes, and causing DNA damage. Prebiotics play a pivotal role in degrading bile salts/acids, thus plummeting their reuptake and enhancing their elimination rate within the intestines[25].

3.5 Utilizing Prebiotics as Protective Agents in Probiotic Formulations

In the probiotic manufacturing process, it is customary to transform them into powdered form via either freeze-drying or spray-drying techniques. Nevertheless, the freeze-drying process is susceptible to several variables that may result in bacterial mortality. To counteract this issue, a protective agent can be incorporated to modify the probiotics' conditions during the freeze-drying process, thereby mitigating cell damage[26].

Prebiotics stand out as a frequently employed safeguard in this context. A group of researchers delved into the potential of protein-trehalose as a protective agent, revealing its substantial influence on the viability of probiotic *Lactobacillus plantarum* TISTR 2075 cultivated in the Plai-Ngahm-Prachinburi rice extract. This protective agent notably bolstered the strain's survival rate post-freeze-drying, achieving an impressive 98.13%. In comparison to alternative protective agents, even during extended storage periods, the protein-trehalose protector continued a substantial population of viable cells, and the bottommost recorded cell mortality rate[27].

Table 1. Mechanism of promoting probiotics by prebiotic intake[15,27]

S. No.	Mechanism of Promoting Probiotics	Advanced Mechanism of Promoting Probiotics
1	Prebiotic foster the proliferation and expansion of probiotic microorganisms	Prebiotics convert into carbon reservoirs as the availability of carbon sources is essential for the proliferation of probiotic microorganisms.
2	Prebiotics enhance the metabolism of probiotics	Through SCFAs generation.
3	Strengthening probiotic resistance to ROS and bile salts through prebiotics	Resistance to ROS by production of butyric acid as a part of SCFA metabolism.
4	Prebiotics working as protective agents in probiotic formulations	Protect bacterial mortality by mitigating cell damage. e.g.- Protein-trehalose.

4. Mechanism of Immune Modulation by Probiotic Bacteria

One crucial role of probiotics is their capacity to regulate the immune system. The oral administration of various probiotic strains, including *Acidophilus, Rhamnosus, Delbrueckii subsp., Bulgaricus, Lactobacillus casei, Plantarum, Lactis,* and *Streptococcus thermophilus* have been observed to increase the population of IgA-producing cells in the intestines in a dose-dependent manner. Probiotics trigger the clonal expansion of B cells, resulting in heightened secretion of IgAs, while simultaneously maintaining the count of CD4+ T cells. Additional studies have uncovered that probiotic bacteria can

induce the secretion of secretory IgA. These IgAs are abundantly released into the intestinal lumen, serving as a defense mechanism to prevent detrimental bacteria from accessing the intestinal epithelium, thereby restricting gut colonization. The rise in IL-6 levels, triggered in a TLR2-dependent fashion, has been recognized as the primary reason for the heightened presence of intestinal IgA-producing cells, without a simultaneous increase in CD4+ T-cell counts[28]. Furthermore, probiotics possess the ability to inhibit the attachment and growth of harmful pathogens on the mucosal layer, thus protecting the intestinal enterocytes and the lamina propria[29].

The existing information indicates that the makeup of the gut microbiome may influence the immune system's response to vaccination. In particular, it has been noted that oral vaccines can disturb the equilibrium of the intestinal microbiota, resulting in either dysbiosis or a reduction in the beneficial microorganisms crucial for supporting effective immunity. Research using an animal model demonstrated a robust association between the activation of Toll-like receptor 5 (TLR-5) and the introduction of a flagellated strain of *E. coli*. Interestingly, this was linked to an improved immune reaction to a deactivated influenza vaccine. These findings underscore the positive influence of probiotic bacteria on enhancing the effectiveness of vaccines[30].

4.1 Immunomodulatory Mechanisms of Action of Various Probiotic Bacteria

The specific interplay among the immune system within the intestines, epithelial cells, and probiotic microorganisms has the capacity to release cytokines with both pro-inflammatory and anti-inflammatory properties. This cascade, in turn, has the potential to finely regulate immune function. Probiotics can, therefore, act as catalysts for an innate, nonspecific immune response, wherein local innate immune cells are triggered to detect infections or tissue damage. Probiotics can exert their influence on the immune response either directly or indirectly, by inciting the production of various cytokines, including Transforming Growth Factors (TGFs), Tumor Necrosis Factors (TNFs), Interleukins (ILs), Interferons (IFNs) and chemokines, through immune cell populations, including Dendritic Cells (DCs), lymphocytes, granulocytes, macrophages, intestinal epithelial cells or mast cells[31]. This intricate process is illustrated in Figure 1.

Lactobacillus can manifest diverse effects, such as strengthening regulatory T cell activity: it prompts Treg cells to generate crucial factors like TGF-β, IL-10, and IL-8, which play a pivotal role in suppressing immune responses. Facilitating IgA production: through a TLR-2 pathway, it increases IL-6 secretion, leading

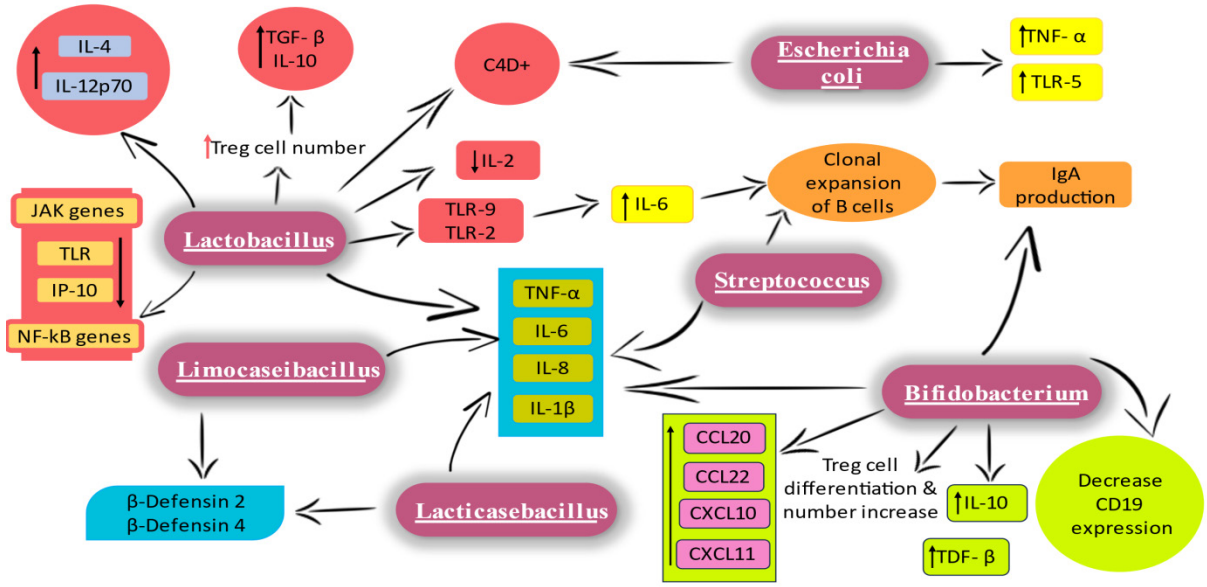

Figure 1. Immunomodulatory mechanisms employed by diverse probiotic microorganisms.

to the expansion of B cells responsible for producing IgA antibodies. This effect is further heightened by the upregulation of CD206, a macrophage receptor integral to antibody processing. Fine-tuning immune signaling: *Lactobacillus* mitigates the activity of JAK/NF-κB pathways, commonly associated with inflammation, while simultaneously promoting the release of IL-12p70 and IL-4—cytokines with diverse immune functions. Modulating co-stimulatory signals: It downregulates the expression of TLRs, which can trigger immune responses, while upregulating CD40 and CD80—co-stimulatory molecules crucial for B cell activation. Regulating proinflammatory signals: *Lactobacillus* degrades IP-10, a potent proinflammatory chemokine, contributing to a more balanced immune response. Enhancing innate immune response: It amplifies the expression of TLR-9, NLRP3, Caspase-1, and IL-18 — key components of the innate immune system's response to various threats. This multifaceted strategy aims to precisely modulate the immune system for the desired effect, emphasizing the intricate interplay between different immune cell types and signaling pathways[32].

Lacticasei bacillus and *Limocasei bacillus* strains have the capability to prompt the expression of β-defensins 2 and 4, as well as IL-8. However, it's worth noting that different research studies have presented conflicting findings regarding the expression of TLRs.

Bifidobacterium can perform the following functions: (i) Inhibit the activity of JAK and NF-κB genetic expression; (ii) Promote the excessive production of IL-10 and TGF-β, while simultaneously stimulating the generation of IgAs; (iii) Promote the differentiation of Treg cells; (iv) Increase the overall numbers of helper (CD4+) and activated (CD25+) T lymphocytes, along with natural killer cells; (v) Reduce the expression of CD19 on B cells; (vi) Induce the generation of monocyte chemoattractant protein 1 and TNF-α by activating TLR-9; (vii) Augment the number of Foxp3(+) T regulatory cells and the secretion of CCL20, CCL22, CXCL10, and CXCL11.

Escherichia coli has the capacity to stimulate the activation of TLR-5 and the production of TNF-α, while also elevating the count of CD4+ cells.

Bacteroidales induce the release of IL-6 while simultaneously increasing the expression of mucin-2 and claudin-1. On the other hand, *Limocasei bacillus, Lactobacillus, bifidobacterium*, *Lacticasei bacillus,* and *streptococcus* have the potential to promote the release of TNF-α, IL-1β, and IL-6. Moreover, *Streptococcus* has the capability to induce the clonal expansion of B cells, stimulating their secretion of IgAs[33].

5. Probiotic Interaction with Various Pathogens

The protective function of probiotic microorganisms against gastrointestinal pathogens has garnered significant interest, particularly because these interactions are a key criterion in the selection of new probiotics for human consumption. The mechanisms involved in host-bacteria interactions include the physical connections between bacteria and the epithelium, bacterial interactions with the immune system, as well as interactions between different bacterial species. Given the intricate nature of the intestinal microbiota, it may be necessary to use combinations of probiotics to address deviations in the microbiota occurring at various locations within the intestinal tract[34].

5.1 Probiotic and Virus

The growing menace of viruses poses an escalating threat to human well-being. The extensive serological diversity within the virus population presents a formidable challenge when it comes to developing vaccines and pharmaceuticals for preventative purposes. Clinical intervention investigations have revealed that specific strains of probiotics may possess the potential to curtail the duration of certain viral infections or mitigate the risk associated with them. Notable examples include *Lactobacillus casei Shirota*, *Lactobacillus rhamnosus* GG, *Lactobacillus reuteri,* and *Bifidobacterium lactis* Bb-12, which have demonstrated the ability to shorten the length of viral diarrhea by approximately 1-1.5 days. Moreover, they have been shown to reduce the likelihood of viral diarrhea, particularly among infants and children, particularly in cases involving rotavirus[35].

When viruses come into contact with mucosal surfaces, they must navigate through three primary defense layers: the protective mucus layer, innate immune defenses, and adaptive immune responses. The mechanisms responsible for countering viral

threats, as outlined in Figure 2, encompass both direct and indirect actions. These comprise (1) Reinforced enhancement of mucosal barrier activity; (2) Production of antiviral antimicrobial peptides (AMPs); (3) Prevention of attachment of the virus to host cells and (4) Adjustment of innate and adaptive immune cell functions to combat viruses[36].

Probiotics combat viral infections, likely due to their capacity to perform several key functions, which include: the exclusion of viruses, reinforcement of the tight connections between enterocytes, the production of antimicrobial agents that may also possess antiviral properties, the stimulation of the immune defenses of host cells, and the binding and neutralization of rotaviruses. Multiple *in vitro* and clinical studies have provided evidence of probiotic bacteria effectively excluding bacterial pathogens[37].

5.2 Probiotics and Fungi

Mycotoxins are secondary compounds generated by microfungi, and they possess the potential to induce diseases and even fatalities in both humans and other animals[38]. These mycotoxins are commonly detected in products like nuts, corn, rice, and various cereals, with contamination occurring either during the cultivation process or during storage. The three primary genera of fungi that produce mycotoxins are *Aspergillus, Penicillium, Fusarium*, and the principal mycotoxins found in food items include aflatoxins, ochratoxins, fumonisins, trichothecenes, and zearalenone.

Certain strains of LAB have demonstrated remarkable effectiveness in eliminating AFB1 in experimental setups. AFB1, the most toxic member of the aflatoxin family, is produced by the fungus *Aspergillus flavus*. It appears that AFB1 attaches to the exterior of both *Lactobacillus rhamnosus* GG and *Lactobacillus rhamnosus*[39]. This binding property appears to persist even after exposure to gastric digestion.

In laboratory experiments using cell lines, it was noted that pre-incubating probiotics with mycotoxins led to a reduction in the adherence of the probiotic-mycotoxin complex with mucus. Conversely, pre-incubating probiotics with mucus decreased the quantity of attached mycotoxin. Certain strains demonstrated increased AFB1 binding upon exposure to bile salts. Furthermore, experiments using Caco-2 cells indicated that probiotics might mitigate AFB1 transport and its harmful effects. They were also shown to protect against AFB1-induced declines in transepithelial resistivity and destruction of DNA. In animal trials, administering probiotics orally along with mycotoxins was linked to elevated fecal excretion

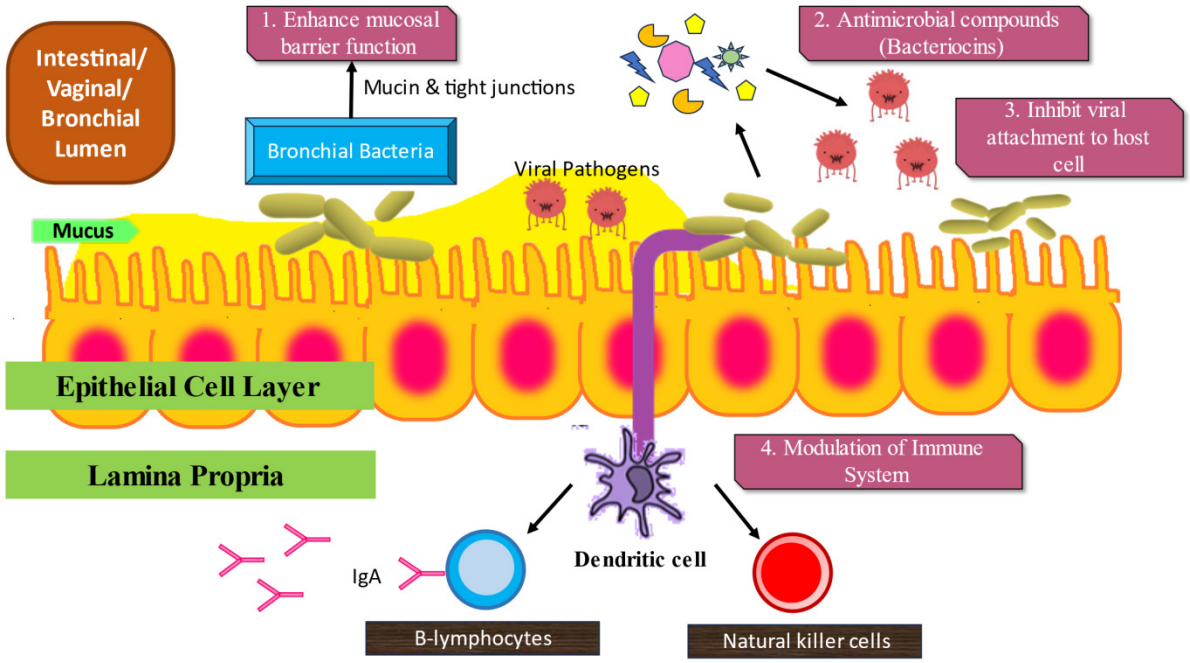

Figure 2. Mechanisms within the microbiome that counteract viral infections.

of mycotoxins, defense against oxidative stress, DNA damage, alleviation of hepatic injury, decrease in tumor incidence, and maintenance of many factors related to the welfare of animals[40].

5.3 Probiotics and Water Pathogens

Toxins produced by cyanobacteria pose a potential threat to the safety of drinking water. Among these toxins, microcystins are hepatotoxins composed of a cyclic heptapeptide, produced by several species of freshwater cyanobacteria genera, including Microcystis, Nostoc, Anabaena, and Planktothrix. These cyanobacteria are found in eutrophic waterbodies worldwide. The most widespread and highly toxic form is microcystin-LR, which includes the amino acids leucine (L) and arginine (R) at specific positions[41].

Human exposure to microcystins can occur when consuming drinking water sourced from contaminated reservoirs, which may contain these toxins. Probiotic bacteria have the capability to eliminate cyanobacterial toxins from aqueous solutions[42]. Research efforts have been primarily concentrated on studying the ability of probiotic bacteria to remove toxins and assessing their potential for purifying water. Among the 11 tested strains, specific probiotic bacterial strains, such as *Bifidobacterium longum* 46, *Lactobacillus rhamnosus* strains GG and LC-705, *Bifidobacterium lactis* 420 and *Bifidobacterium lactis* Bb12, have demonstrated the highest effectiveness in removing the cyanobacterial peptide toxin microcystin-LR[43]. The most effective toxin elimination took place at a temperature of 37°C across all five strains. This elimination process was remarkably rapid, with around 80% of the cyanotoxins being removed within 24 hours of the incubation period. Supplementing bacterial cells with glucose as a nutrient source additionally enhanced the removal of toxins[44]. The precise processes by which probiotic microorganisms eliminate toxins are currently unknown, However, present evidence suggests that microcystins may be broken down enzymatically in the vicinity of actively metabolic probiotic bacteria[45].

6. Engineering Probiotics for Improving Human Health

Engineered probiotics represent a novel class of microorganisms created by altering native probiotics through genetic editing techniques. As tools and technological advancements continue to evolve, the possibilities for engineering improvements in probiotics are becoming increasingly diverse and attainable. Due to advancements in metabolic engineering and synthetic biology, the field of probiotics engineering has now reached a stage where it holds the promise of designing microorganisms with the capability to selectively target specific tissues and cells, rather than exerting a broad impact on the entire organism. This approach allows for the creation of innovative probiotics with customized characteristics and functionalities[46].

Engineered probiotics are finding applications as therapeutic instruments for delivering molecules to eukaryotic cells in various medical conditions. Enzymes hold a pivotal position in cellular metabolism, facilitating intricate biological processes necessary for sustaining life. When an enzyme is absent or malfunctioning, it can lead to metabolic disorders. Therefore, the administration of enzyme replacements to reestablish proper metabolism or the removal of toxic substances and the inhibition of their production show significant promise as therapeutic approaches for addressing metabolic disorders[47].

Contemporary investigations have delved into an innovative strategy for addressing Alzheimer's disease, focusing on the deployment of a *Lactobacillus lactis* strain harboring a plasmid (pExu) housing a eukaryotic expression cassette encoding the human p62 protein. Upon oral administration to 3xTg-AD mice over a period of two months, these microorganisms elicited an elevation in endogenous p62 levels within the brain. The delivery mechanism of this protein involves both lymphatic vessels and neural endings, yielding positive effects on pivotal characteristics associated with Alzheimer's disease. The mice exhibited improved cognitive function, alterations in the ubiquitin-proteasome system and autophagy, diminished concentrations of amyloid peptides, and attenuated neuronal oxidative and inflammatory processes[48].

A group of investigators genetically modified a human gut strain identified as *Lactococcus lactis* with the objective of addressing type 1 Diabetes. This genetically altered *L. lactis* exhibited the capacity to release both the complete proinsulin autoantigen and the biologically active immunoregulatory cytokine IL-10. To assess the effectiveness of this approach, they orally administered

recombinant *L. lactis* along with a low dose of a non-specific immune modulator called anti-CD3 to non-obese diabetic mice. The results demonstrated that 59% of the murine models, corresponding to 36 out of 61 mice, underwent a persistent reversal of autoimmune diabetes and a reduction in inflammation within the pancreatic islets when compared to the control group[49].

6.1 Engineering Probiotics for Disease Diagnosis and Detection

Modifying bacteria to detect crucial molecules within the body and subsequently generate specific signals empowers probiotics to serve as diagnostic tools. One frequently employed approach involves integrating a quorum sensing system into probiotics to detect pathogens causing infection. For example, a diagnostic circuit was established within *Lactococcus lactis* to facilitate the on-site detection of a molecule produced by *Vibrio cholerae*. This novel system facilitates the early identification of cholera infection, indicated by a discernible alteration in color within the fecal samples of the host. In this investigation, a pioneering apparatus for sensing signal molecules was developed by amalgamating the transmembrane ligand-binding domain of CqsS sourced from *Vibrio cholerae* with the signal transduction domain of NisK obtained from *L. lactis*. Upon detecting the signaling molecule produced by Vibrio cholerae, the genetically modified *L. lactis* instigated the secretion of β-lactamase, resulting in a discernible color change when exposed to nitrocefin. The oral delivery of this genetically engineered *L. lactis* to mice afflicted with cholera elicited affirmative signals in their fecal samples[50].

Beyond identifying pathogenic infections, probiotics integrated with sensors possess the capability to produce observable signals for the diagnostic assessment of cancer. The oxygen-deprived and necrotic zones within tumors provide favorable conditions for certain anaerobic bacteria like *E. coli, Salmonella,* and *Clostridium*. These bacteria's inherent tendency to thrive in tumor environments allows them to specifically target tumors and their metastases[51]. A group of researchers devised a diagnostic tool by modifying a probiotic strain of *E. coli* Nissle 1917 (EcN), a non-pathogenic strain of *E. coli*. This engineered strain was tailored to identify liver metastasis in mice and produce a discernible signal in urine. In this setup, *E. coli* was used to simultaneously initiate the expression cassettes for luciferase (luxCDABE) and β-galactosidase (lacZ). With this configuration, the newly created probiotic strain known as PROP-Z was able to provide a luminescence signal in addition to a colorimetric readout. In order to enhance the efficacy of PROP-Z, a toxin-antitoxin network, and the Bacillus subtilis gene dlp7 were integrated. The cells were stimulated by these inclusions to preserve the plasmid and divide it uniformly throughout the cycle of cell division. When PROP-Z was orally administered along with luciferin/galactose conjugate, it led to the release of luciferin into the bloodstream. This luciferin could then be excreted by the kidneys, enabling its detection in urine samples from the mice[52].

Probiotics can also be customized to detect inflammation. Nitric oxide (NO) functions as a signaling molecule, typically serving as an anti-inflammatory agent during standard physiological settings. However, excessive NO production in abnormal situations can lead to inflammation, harm to tissues, and even malignancy. Consequently, elevated NO levels serve as an indicator of inflammation in conditions like inflammatory bowel diseases. A group of scientists developed a live bacterial sensor system for the detection of nitric oxide as an indicator of inflammation in the intestines. Using this synthetic system, they were able to modify a strain of *E. coli* such that it could recognize the generation of nitric oxide within the gastrointestinal tract. The existence of NO-induced the expression of a DNA recombinase, leading to the enduring activation of a DNA switch that could be passed on to the bacterial offspring following cell division[53].

A different noteworthy tiny compound is tetrathionate. Bacteria that are able to reduce sulfate can form hydrogen sulfide (harmful to host cells) in the intestinal environment. Elevated levels of hydrogen sulfide have the ability to inhibit cytochrome c oxidase, which in turn prevents butyrate from being oxidized inside the colon's epithelial cells. Hydrogen sulfide has the ability to detoxify the host, resulting in the production of thiosulfate. Then, during bouts of intestinal inflammation, ROS oxidizes thiosulfate into tetrathionate[54]. Hence, tetrathionate has the capability to function as a biomarker for the detection of gastrointestinal tract diseases. Tetrathionate may

be used as a terminal acceptor of electrons by some pathogenic bacteria, which gives them a competitive edge in growth. A two-component regulatory system (TCRS) is present in these bacteria, and its purpose is to detect tetrathionate and then reduce its build-up. Leveraging this mechanism, a team of researchers engineered a probiotic strain of *E. coli*. This strain integrates TCRS from *Salmonella* to detect and memorize information about tetrathionate exposure throughout the gastrointestinal tract. This technique was designed by the researchers to activate the *cI/Cro* genetic element from phage lambda[55].

7. Approaches for the Synthesis of Antimicrobial Peptides using Probiotics

AMPs, sometimes referred to as host defense peptides, are synthesized by a diverse array of organisms as a means of providing defense against pathogenic bacteria and diminishing competition within their ecological niches[56]. These peptides are typically short in length, often carrying a positive charge, and can be generated through either ribosomal mRNA translation or non-ribosomal protein synthesis[57]. For the recombinant production of AMPs in bacteria, specific prerequisites are common to fundamental expression systems. AMPs have been synthesized as fusion proteins utilizing diverse fusion partners or by encoding the complete natural secretion apparatus of the particular AMP within the bacterial host[58-60]. The transportation of AMPs to the gastrointestinal tract has been accomplished through the expression and liberation of these agents from probiotics.

This has been achieved by using fusion proteins in conjunction with secretion signals[60] and by causing the probiotic host to lyse in order to release the peptides that are held inside the bacteria[61]. Another crucial aspect in the engineering of probiotics involves the imperative to formulate strategies for biocontainment. An AMP-producing probiotic released into the environment could potentially trigger various detrimental consequences, including the promotion of antibiotic resistance or ultimately inducing to dysbiosis in other species. Consequently, it is imperative to incorporate biocontainment measures when engineering genetically modified organisms[62]. The two main categories of these containment techniques are passive and active methods. Passive mechanisms involve the excision of genes encoding necessary metabolites or amino acids, which are then externally supplied to sustain bacterial growth[63]. Conversely, active mechanisms involve the utilization of more intricate genetic circuits that can sense environmental signals, either the presence or absence thereof, to activate a circuit or instigate a self-destructive mechanism[64].

EcN has undergone modifications to facilitate the synthesis of the antimicrobial peptide HBD2, as illustrated in Figure 3 (a), with the objective of addressing Crohn's disease. The strong and activated T7 promoter regulated the production of the AMP, which was extracted using a fusion protein with YebF as the fusion partner. YebF possesses the capability to translocate peptides attached to its C-terminus outside of the cell towards the surrounding media in both laboratory strains of *E. coli* and EcN[65]. This fusion protein was efficiently secreted, and HBD2 exhibited antimicrobial efficacy[66].

Segments responsible for detecting and generating therapeutic compounds within *E. coli* can be organized into distinct units through the implementation of the "sense-kill" system. This approach is dependent on a sensing module that consistently produces the transcription factor LasR. LasR subsequently engages with the quorum-sensing molecule homoserine lactone (HSL) derived from *P. aeruginosa,* activating the therapeutic modules regulated by the PluxR promoter 200 (which becomes active upon forming the LasR-3OC12HSL complex)[67]. The inaugural investigation utilizing this mechanism encompassed the transcriptional activation of two components prompted by HSL-LasR: the antimicrobial peptide pyocin S5 and the lysis E7 protein When the concentration of E7 was reached in the presence of HSL, the formation of pyocin S5 and the E7 protein occurred. This threshold concentration triggers the chassis to undergo lysis, culminating in the liberation of the antimicrobial peptide into the surrounding milieu, with a specific targeting effect against *P. aeruginosa*[68]. This mechanism was subsequently effectively implemented in an EcN chassis, and its antimicrobial efficacy was substantiated through experiments conducted with *Caenorhabditis elegans* and mice[61].

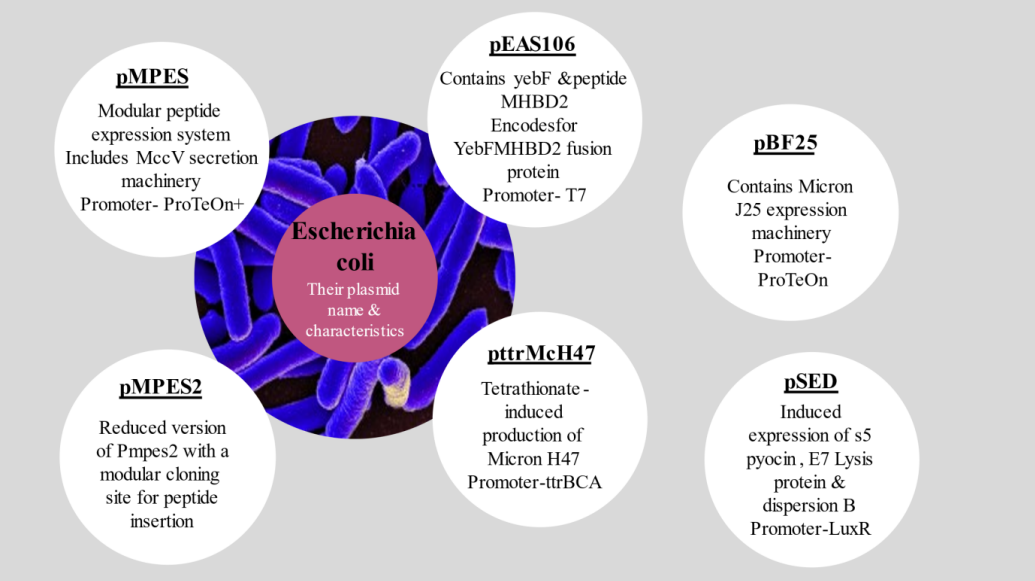

Figure 3. Designing plasmids to enable probiotic bacteria to synthesize AMPs. (a) *Escherichia coli* Nissle (b) Lactic acid bacteria.

It is thought that consuming LAB is harmless for humans and is often categorized as "food-grade" microorganisms[69]. These bacteria naturally generate a class of AMPs called bacteriocins as illustrated in Figure 3 (b). Among LAB, Lactobacillus and *Lactococci* have received the most extensive research attention[70]. A recent work employed the pAMJ165 plasmid to facilitate the heterologous development of a new hybrid AMP called cLFchimera in *L. lactis*. This AMP incorporates both lactoferrampin and lactoferricin[71]. The lactic acid-inducible p170 promoter governs the production of proteins in this plasmid, and low pH causes overexpression of this promoter[72]. The signal peptide sp310 mut2 is used for peptide secretion. Although *L. lactis* normally contains this signal peptide, it underwent genetic modifications to enhance

its efficiency in promoting secretion. Employing this method, the secretion of the peptide was achieved successfully, and the peptide exhibited antioxidant, antibiofilm, and antibacterial properties when tested *in vitro* against various foodborne pathogens[73].

8. Conclusion

This study highlights the critical role that probiotics play in reducing microbial problems by focusing on their interactions with waterborne pathogens, mycotoxins, and fungus. The data presented underscores the potential of specific strains of LAB in mitigating fungal infections induced by mycotoxins. Notably, LAB demonstrated effectiveness in binding and reducing the adhesion of AFB1, a potent toxin produced by *Aspergillus flavus*. Moreover, probiotics exhibited promising outcomes in experimental setups, showing protection against AFB1-induced damage and promoting excretion. In the context of water safety, engineered probiotics exhibit significant promise in combating toxins generated by cyanobacteria, particularly microcystins. Certain strains of LAB, including *Lactobacillus rhamnosus* GG and *Lactobacillus rhamnosus* LC-705, demonstrated high efficacy in eliminating microcystin-LR, a prevalent and highly toxic variant. The quick elimination of toxins, which was seen after incubation for 24 hours at 37°C, offers a strong basis for more investigation. The emergence of engineered probiotics opens new frontiers in tailored therapeutic interventions. Studies presented here exemplify their potential in addressing complex conditions, such as Alzheimer's disease and Type 1 diabetes. Engineered strains effectively delivered therapeutic molecules, inducing positive outcomes in mouse models. This signifies a promising avenue for precision medicine, where probiotics can be customized to target specific tissues and cells, revolutionizing treatment strategies. Furthermore, the integration of sensing mechanisms within probiotics holds immense diagnostic potential. From detecting cholera infection to signaling inflammation or even identifying biomarkers for cancer, engineered probiotics offer a non-invasive and innovative approach to disease diagnosis. These developments represent a paradigm shift in healthcare, potentially revolutionizing early disease detection and monitoring. As research advances, the production of antimicrobial peptides using probiotics is poised to play a pivotal role in combating pathogenic bacteria. The study of AMPs, their production, and delivery using probiotics demonstrate a multifaceted approach to address microbial threats. Biocontainment strategies are crucial to ensure the responsible deployment of AMP-producing probiotics.

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