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The Emerging Role of Engineered Probiotics in Gastrointestinal Health: Mechanisms, Challenges, and Future Perspectives

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Abstract

Traditional probiotics, such as *Lactobacillus*, *Lactococcus*, and *Bifidobacteria*, defined as live microorganisms used to manage gastrointestinal disorders such as inflammatory bowel disease (IBD) and irritable bowel syndrome (IBS), have demonstrated variable clinical efficacy. The advent of synthetic biology has ushered in a paradigm shift, enabling the rational design and production of Next-Generation Probiotics (NGPs) or engineered strains with precise therapeutic and diagnostic functionalities at targeted gastrointestinal sites. This review discusses predominant molecular mechanisms employed by engineered probiotics, including enhanced biosynthesis of bioactive metabolites like short-chain fatty acids (SCFAs) and site-specific delivery of immunomodulatory cytokines such as interleukin-10 (IL-10). Additionally, critical biosafety considerations related to biocontainment and horizontal gene transfer (HGT) are evaluated, highlighting advanced genetic safeguards such as CRISPR-Cas–based kill switches to mitigate dissemination risks. Regulatory frameworks governing these live biotherapeutic products (LBPs) are also analyzed. Future directions encompass the integration of state-of-the-art gene editing technologies and GI biosensors for in vivo disease monitoring, as well as the potential for engineered probiotics in personalized medicine. Collectively, advancements in synthetic biology and genomics have propelled engineered probiotics as programmable live therapeutics, offering novel avenues for the prevention, diagnosis, and treatment of refractory gastrointestinal diseases. These approaches, while navigating biosafety and regulatory challenges, promise enhanced clinical efficacy and pave the way for precision microbiome interventions.

1. Introduction

The definition of probiotics has been established by settled terminology, consisting of live microbial agents that, in adequate dosages, can provide beneficial health impacts on the health-conditioned host. The targeted microbial strains, over the past twenty years, have received immense attention, and their importance in reinforcing the general immune system and regulating gut homeostasis has been aptly defined. Although conventional probiotics are in widespread use, they demonstrate inefficient and highly conditionally dependent functional properties that are highly dependent on gut conditions and intestinal microbial communities (Iacomino *et al*., 2024; Chandrasekaran *et al*., 2024).

The current focus in academic literature has moved on to NGPs, which are designer bacterial strains synthesized using the techniques of synthetic biology. These probiotics are specifically designed, using highly advanced genetic techniques, for carrying out specialized therapeutic and diagnostic functions, going beyond what traditional probiotics might allow. NGPs intend to bridge the gap that exists between small-molecule therapies and microbiome therapies using novel molecular mechanisms capable of targeted interventions. Specifically designed strains of NGPs, including *Bacteroides fragilis*, *Akkermansia muciniphila*, and *Faecalibacterium prausnitzii*, have shown promise in demonstrating immunomodulatory and anti-inflammatory effects in preclinical and clinical settings (Al-Fakhrany & Elekhnawy, 2024). The designer nature of these strains enables them to interact specifically with the host microbiome, potentially improving metabolic and immune functions and offering beneficial applications for chronic gastrointestinal and systemic diseases (Al-Fakhrany & Elekhnawy, 2024). The gastrointestinal tract is home to a complex microbial population consisting of many microbial communities, the role of which in various biological functions has been abundantly confirmed. These processes include metabolic function, immune function modulation, and integrity of

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epithelial barriers, among others. An unbalanced microbial ecosystem, denoted by 'dysbiosis,' plays a central pathogenic role in several major chronic diseases, such as IBD, IBS, and obesity, and also has a significant association with several systemically linked diseases, such as osteoporosis (Chandrasekaran *et al*., 2024; Latif *et al*., 2023; Shen *et al*., 2022). Through genetic engineering, NGPs can serve as LBPs that possess the ability to produce and secrete therapeutic proteins directly at the site of inflammation.

This will enable the localized production of a dosage form of biological drugs (such as cytokines) without any systemically induced side effects, unlike pharmacological therapy (Rottinghaus *et al*., 2020; Zhang *et al*., 2023). The application of NGPs, serving as LBPs, is a cutting-edge technology for the treatment and therapy of any disease that has an inflammatory or metabolic cause (Zhang *et al*., 2023). The emergence of recent advancements in high-throughput sequencing, genome editing tools like the CRISPR-Cas system, and computational modeling has led to the discovery and use of NGPs in new and exciting ways. Different from traditional probiotics, the current trend among researchers and analysts reveals that next-generation probiotics are also increasingly designed and constructed using advanced and complex synthetic biological approaches that make it easy to align the genetic and metabolic properties.

For example, the use of AI technology in strain selection and the integration and application of the CRISPR Cas system in genome editing and modeling allow for the easy and fast discovery and programming of therapeutic properties and interventions relevant to beneficial microbes. This current version permits the design and construction of the next-generation probiotics to not only improve the capacity to colonize and interact but also to meet biosafety and regulatory standards by the addition and integration of fail-safe biological circuits (Tiwari *et al*., 2024).

2. Mechanisms of action of engineered probiotics in the gastrointestinal tract

Engineered probiotics exert their therapeutic effects not only by augmenting the intrinsic pathways of parental strains but also by integrating synthetic biochemical circuits and heterologous genetic modules. The pharmacological profile and therapeutic efficacy of these microorganisms are fundamentally dictated by the specific engineering strategy employed. For instance, metabolic pathway enhancement, such as the up-regulation of genes involved in the biosynthesis of SCFAs or indole-based tryptophan metabolites, is strategically tailored for chronic interventions.

These approaches aim to achieve sustained, local homeostatic shifts within the intestinal microenvironment through the continuous production of small-molecule metabolites. Conversely, recombinant protein secretion (e.g., the delivery of therapeutic cytokines like IL-10 or targeted neutralizing antibodies) is often prioritized for acute clinical manifestations. While these proteins can be engineered for high-affinity local action to minimize off-target effects, they can also be optimized for mucosal absorption to exert systemic immunomodulatory influences.

As illustrated in Figure 1 and synthesized in Table 1, these engineered systems demonstrate superior mechanistic advantages over conventional probiotics, offering precise spatiotemporal control over the delivery of therapeutic payloads.

This figure illustrates the distinct functional pathways exhibited by traditional and engineered probiotics within the gastrointestinal tract.

Traditional probiotics enhance epithelial barrier integrity through upregulation of mucin, occludin, and claudin-1, modulate host immune responses via cytokine secretion, and contribute to endogenous production of SCFAs.

Table 1. Comparative analysis of wild-type and engineered probiotics: mechanisms, pharmacological outcomes, and therapeutic applications

Mechanism / Engineering Approach	Traditional Probiotic (Wild-Type)	Next-Generation Probiotic (NGP)	Pharmacological Profile (Reviewer's Requirement)	Therapeutic Example	References
Metabolic Pathway Optimization (e.g., SCFAs)	Dependent on environmental and dietary precursors; stochastic and low-yield output.	Orchestrated, high-yield metabolite production via synthetic biosynthetic modules and pathway rewiring.	Chronic / Sustained: Continuous local homeostatic modulation with long-term metabolic reprogramming.	*E. coli* Nissle (*EcN_TL)* engineered for enhanced butyrate and propionate production.	Guo *et al*., 2023
Heterologous Protein Secretion (e.g., IL-10)	Basal, non-specific immunomodulation restricted to native regulatory circuits.	Targeted secretion of defined therapeutic payloads (e.g., IL-10) triggered by inflammatory cues.	Acute / Directed: Rapid attenuation of inflammation with potential systemic exposure via mucosal interfaces.	*Lactococcus lactis* engineered to secrete IL-10 for inflammatory bowel disease management.	Pham *et al*., 2024; Zhang *et al*., 2023
Pathogen-Specific Intervention	General competitive exclusion and broad, non-specific antimicrobial activity.	Programmed biosensing coupled with site-specific secretion of targeted antimicrobials.	Acute / Prophylactic: Transient, site-specific pathogen decolonization with minimal microbiota disruption.	Engineered probiotic strains for targeted inhibition of *Clostridioides difficile*.	Mazziotta *et al*., 2023

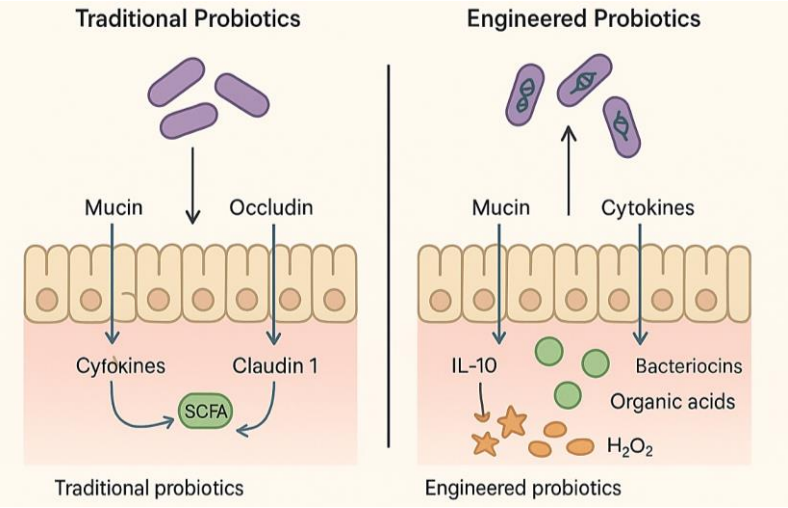

Figure 1. Comparative mechanisms of action of traditional and engineered probiotics in the gastrointestinal tract

In contrast, engineered probiotics incorporate advanced genetic and metabolic modifications that augment SCFA biosynthesis (propionate, acetate, and butyrate), enable targeted IL-10 delivery for localized immune regulation, and provide enhanced pathogen inhibition through competitive adhesion, nutrient competition, and secretion of antimicrobial compounds, including bacteriocins, organic acids, and hydrogen peroxide.

2.1. Targeted therapeutics: From metabolic biosynthesis to precision immunomodulation

One of the core examples under the paradigms of probiotic engineering is the metabolic promotion of important metabolites, specifically SCFAs like acetate, propionate, and butyrate, which are known to be depleted in the intestine due to dysbiosis and other pathologies like IBD (Fusco *et al*., 2023; Liu *et al*., 2024). *Escherichia coli Nissle 1917* (EcN) has been considered the best model for these approaches, due to its highly manipulable genome that allows for easy chromosomal integration, its safety record for the last century, and its effective colonization properties in the human intestine (Rottinghaus *et al*., 2022; Seco & Fernández, 2022).

Recent advances have moved beyond simple overexpression and now involve complex heterologous biosynthetic pathways. It is important to note that the engineering of the butyrate biosynthesis operon (But operon) allows for high-yield anaerobic butyrate production in strains such as *EcN_TL*. This approach was recently demonstrated to correct local homeostasis, as it strongly increases systemic plasma SCA concentrations, conferring cardioprotection against myocardial IR injury (Guo *et al*., 2023). These studies revolutionize the role of the gastrointestinal tract as a biomanufacturing site for systemic therapeutics, establishing a gut-organ axis. Second, therapeutics based upon NGPs have grown from a generalized biodefense paradigm. Recent approaches for their design focus upon delivery mechanisms for particular bioactive molecules, shifting from generalized antioxidant delivery for generic biodefense strategies to targeted biodefense strategies as enzymatic delivery vehicles for particular enzymes, such as superoxide dismutase (SOD) or catalase, designed for neutralization of reactive oxygen species (ROS). Specific delivery for bacteriocins, such as Microcin V or Colicin, is also possible, which would allow for targeted reduction of pathogenic bacteria with decreased side effects upon commensal flora (Pham *et al*., 2024; Zhang *et al*., 2023). Conventional probiotics have long been appreciated for the mild immune system modulation endowed by the interactions between innate microbe-associated molecular patterns (MAMPs) and Toll-like receptors (TLRs) present in host cells. These interactions might start immune response cascades even in the cells of the intestinal wall and could direct the differentiation of T cells into regulatory cells of a Th2 type and stimulate the production of endogenous anti-inflammatory cytokines, such as IL-4 and IL-10 (Cristofori *et al*., 2021). However, the effectiveness of these interactions is often irregular and cannot be applied to the therapy of inflammatory disease conditions.

NGPs have been engineered to capitalize on and build upon these fundamental processes through the provision of programmatic precision. A classic example within this area is the recombinant *L. lactis* strain engineered for the secretion of human IL-10. The key component of this approach is the direct and localized delivery of the anti-inflammatory cytokine to the site of inflammation within the mucosa, and this has recently been demonstrated within clinical trials for Crohn's disease to be safe and to result in reduced disease activity (Zhang *et al*., 2023). The main advantage of this approach, and one of its notable strengths, is its ability to deliver high concentrations of the drug directly at the site of disease without any potential systemic side effects (Wei *et al*., 2025).

2.2. Engineering enhanced colonization resistance and barrier integrity

Although traditional probiotics depend on stochastic interactions for supporting intestinal health, NGPs have been designed with the objective of enhancing the mucosal surface and overcoming pathogens through four main engineering approaches: Engineered Adhesion: In order to overcome the fleeting nature of probiotics, NGPs are designed to produce synthetic adhesins as well as surface-expressed proteins like MBP. This enables enhanced binding to mucosal sites, thereby providing longer

retention as well as competitive exclusion of enteric pathogens in the mucosal niche (Tiwari *et al*., 2024).

Engineered Bacteriocin Production: In addition to their inherent antimicrobial potential, NGPs can be designed to produce and secrete high-affinity antimicrobial peptides, colicins, and microcins, which can selectively destroy target bacteria (for example, *Clostridioides difficile*) without affecting the beneficial microbiota (Mazziotta *et al*., 2023). Engineered Barrier Fortification: Probiotics may serve as a vehicle for the in situ delivery of trophic agents and structural proteins, including the use of recombinant expression to secrete Transforming Growth Factor-beta (TGF-β) or binding stabilizers that immediately enhance the transcription levels of claudin-1 and occludin to reduce permeability and prevent the passage of endotoxins (Latif *et al*., 2023; Pham *et al*., 2024). Quorum-Sensing Interference: One of the most advanced methods is the design of strains to interfere with pathogen communication, either through the production of enzymes that break down communication molecules or the production of QS antagonists. NGPs can reduce the virulence of pathogenic populations by suppressing their biofilm or toxins (Tiwari *et al*., 2024; Zhang *et al*., 2023).

Altogether, these functional traits cause probiotics to shift from passive competitors to active therapeutics, capable of conferring a strong and reliable degree of colonization resistance, which is superior compared to the intermittent and often low-level competitive ability presented by traditional strains (Tiwari *et al*., 2024; Pham *et al*., 2024). A comprehensive synthesis of these engineering strategies, including their molecular targets, specific chassis examples, and clinical implications, is systematically summarized in Table 2.

2.3. Comparative efficacy of prominent engineered strains: Immunomodulation versus metabolic engineering

Engineered probiotic microorganisms are a flourishing area in targeted microbial therapies for inflammatory and metabolic disorders. The two most studied probiotic strains developed are *EcN_TL*-based strains and Lactococcus lactis strains modified for the secretion of IL-10. A comparison indicates the different modes and levels of efficacy for these two strains (Pham *et al*., 2024; Wei *et al*., 2025). *EcN_TL* mainly addresses the field of metabolic engineering to enhance the yield of desirable metabolites, such as SCFAs, which help to establish the anti-inflammatory response and the integrity of the barriers in the gut. It has been proven that *EcN_TL* effectively attenuates inflammation, safeguards the function of the intestinal epithelial cells, and also confers resistance against ischemic injury, not limited to the intestine (Guo *et al*., 2023; Pham *et al*., 2024; Rizzi, Costa, Delpino, Flores, & Pieniz, 2025). On the other hand, Lactococcus lactis secreting IL-10 acts through localized immune modulation through the direct delivery of the anti-inflammatory cytokine IL-10 in the lamina propria of the gut. Clinical trials have verified the safety and suppression of disease activity in Crohn's disease patients, hence validating the preferability of this localized delivery of cytokines over systemic administration, thereby minimizing side effects (Zhang *et al*., 2023; McKay *et al*., 2011).

Choosing the chassis organism as well as the engineering strategy should be related to the underlying pathology; *EcN_TL* offers systemic metabolic support relevant to chronic or systemic inflammation, whereas *L. lactis*-IL-10 offers precise localized immunomodulation, which is ideally suited to acute intestinal inflammation. Both methods are prone to problems like genetic instability and microbiome-host interactions, thus underlining the requirement to pursue fundamental research with regard to optimization of efficacy and safety (Guo *et al*., 2023; Zhang *et al*., 2023; Pham *et al*., 2024).

3. Developmental challenges, genetic stability, and safety risks of NGPs

Although NGPs have great potential therapeutic value, their design and development as effective agents for therapeutic use are currently slowed by certain challenges. The major issue within the NGP technology is genetic safety. However, the possibility of NGPs driving unintended changes to either the microbiome or the human host cannot be disregarded (Tiwari *et al*., 2024).

Table 2. Representative engineering mechanisms of NGPs and their therapeutic outcomes

Engineering Strategy	Molecular Target / Pathway	Probiotic Strain Example	Targeted Disease / Application	Key Outcome	Reference
Metabolic Augmentation	Butyrate synthesis (*but* operon); SCFA pathways	*EcN_TL*	IBD; Myocardial I/R injury	Increased plasma SCFAs; localized homeostatic shift	Guo *et al*. (2023)
Precision Immunomodulation	Recombinant IL-10 expression and secretion	*L.lactis*	IBD	Localized cytokine delivery; reduced systemic toxicity	Pham *et al*. (2024); Zhang *et al*. (2023)
Targeted Pathogen Inhibition	Colicins; Microcin V; bacteriocin clusters	Engineered *Lactobacillus* / *EcN*	*Clostridioides difficile* overgrowth	Selective pathogen exclusion; preserved commensal flora	Mazziotta *et al*. (2023)
Barrier Fortification	Upregulation of Claudin-1, Occludin; TGF-β delivery	Modified *Bifidobacterium*	Gut barrier dysbiosis	Reduced intestinal permeability; prevention of endotoxin translocation	Latif *et al*. (2023); Pham *et al*. (2024)
Quorum-Sensing Interference	degradation; signal-quenching enzymes	Engineered probiotic chassis	Biofilm-forming pathogens	Quenched virulence; inhibition of pathogen communication	Tiwari *et al*. (2024)

Genetic stability plays a critical role in ensuring that any new strain of probiotics created will retain desirable properties while preventing the development of harmful ones. In this respect, chromosomal integration of an external gene into the bacterial host is preferred over plasmid vectors, which are prone to genetic instability and HGT (Seco & Fernández, 2022). One of the primary issues associated with creating genetically modified probiotics is HGT, or gene transfer among microbes. More specifically, there is a major concern regarding the transfer of a gene or a sequence of DNA linked to engineered probiotics into the commensal microbes of the human gastrointestinal tract or even into opportunistic pathogens. This consideration focuses particularly on antibiotic resistance gene transfer. This issue can bring about a major concern by creating a critical public health crisis due to the development of multidrug-resistant bacteria within the human gastrointestinal tract (Tiwari *et al*., 2024; Seco & Fernández, 2022).

Even if a parental strain has a history of safe usage (for example, *ECN* 1917), genetic modification could potentially activate genes involved in virulence factors and/or acquire new virulence factors. Moreover, a meticulous examination of the engineered microbe's metabolism, as well as its interaction with natural microbiota, is essential to ensure that there are no harmful metabolites. The assessment of safety must be conducted on a strain-by-strain basis, taking into account microbial species and the genetic risks associated with a specific strain (Carolak *et al*., 2025).

3.1. Technical challenges in the gastrointestinal tract: From metabolic fitness to biocontainment

To be therapeutically effective, probiotics should be able to withstand the very low pH and high concentration of bile salts present in the duodenum before reaching their target location in the distal intestines. A significant concern of NGPs is the metabolic burden imposed by the recombinant expression of genetic circuits. A major factor in this is the complexity of function when probiotics are engineered for tasks like the production of anti-cancer compounds or the regulation of the gut microbiome (Thoda & Touraki, 2025; Wang *et al*., 2022).

The process of maintaining high copy-number plasmids and the biosynthesis of biomolecules like recombinant cytokines or multi-step biosynthetic processes for SCFA in bacteria can affect the utilization of vital cellular resources like ATP, amino acids, and translational functions, which in turn can affect the physiological fitness of the bacteria. For example, the bacteria can show a reduced ability to start the process of stress response, which in turn affects the viability of the bacteria compared to wild-type bacteria (Kim *et al*., 2023; Tiwari *et al*., 2024). To counter such fitness costs, novel delivery systems such as encapsulation with stimulus-responsive polymers are being explored. These so-called “smart” materials can enclose the payload and protect engineered bacterial cells from their surroundings, releasing them only upon detecting a specific physiological signal related to pH or inflammation markers. Such a dual approach combining genetic optimization and physical protection is necessary for bacterial stability and selective action in the harsh environment of the GI tract (Kim *et al*., 2023; Wei *et al*., 2022).

Since engineered probiotics have to resist the gastric acidity and bile salt levels found in the duodenum before reaching their target loci in the distal part of the intestine to have therapeutic effects, the metabolic burden exerted on the cells when heterologous genetic networks are expressed remains among the challenges facing Next Generation Probiotics. This is even more true when the strains have complex functions like the production of anti-cancer compounds and gut microbiota regulation (Thoda *et al*., 2025; Wang *et al*., 2022). The maintenance of high copy number plasmids and the ensuing biosynthesis of complex molecules such as cytokines or multi-step SCFA biosynthesis also divert critical resources such as ATP, amino acids, and translation machinery needed by the organism for its own survival processes. These will significantly affect the bacteria’s physiological fitness and render it readily susceptible to any stressor within the gastrointestinal tract, thus potentiating decreased viability and colonization rates as opposed to wild-type organisms (Kim *et al*., 2023; Tiwari *et al*., 2024).

To counteract these costs of fitness, innovative delivery methods incorporating, for instance, encapsulation in stimulus-responsive polymers are currently in development. Such “smart” materials are intended to contain the payload and protect the engineered bacteria from exposure to the surrounding GI environment by releasing them only in response to particular physiological signals (e.g., pH or inflammation markers). Both biological and physical component optimization are necessary to provide biological stability and effective action in the very challenging environment of the gastrointestinal tract (Kim *et al*., 2023; Wei *et al*., 2022).

To limit the unintentional release of designed bacterial strains into the environment, as well as the transfer of genetic material from one organism to another, it is important to design appropriate measures for biocontainment. The biocontainment strategies are designed to ensure that genetically modified microorganisms show limited growth based on specified conditions, such as the gastrointestinal tract of the organism's body (Iacomino *et al*., 2024; Carolak *et al*., 2025). One of the most prominent and cutting-edge uses within this area is the implementation of kill switches based on CRISPR-Cas technology. These kill switches can be designed within bacterial strains, including *ECN 1917,* to self-destruct in response to the presence of an inducer molecule or when they leave their native environment in the intestinal tract (Rottinghaus *et al*., 2022). These not only serve as an effective method to prevent unregulated growth but can also be employed to remove unwanted genetic material by cutting specific DNA sequences, even after bacterial cell death. The emergence and implementation of these methods could thus readily allow for greater usage of NGPs within clinical settings (Dai, 2023).

3.2. Evolving regulatory frameworks: from probiotics to LBPs

With the recent developments in microbiome therapeutics, there now exists a new kind of LBP requiring

specific regulation. The FDA has outlined specific criteria to regulate LBPs, focusing on Chemistry, Manufacturing, and Controls (CMC) to guarantee the stability of the genetic matter, the absence of contamination, and the functionality of the final product. It is worth mentioning that, related to this issue, the FDA document "Early Clinical Trials with Live Biotherapeutic Products: Chemistry, Manufacturing, and Control Information" points to the need for specific information related to the efficacy, quality, and safety related to the specific features of LBPs, and not not just an adjustment to the criteria applied to common medications or typical probiotics. It is also clear that recent FDA approvals for microbiome therapeutics point to the fact that the process, though hard, is now clear and accessible for well-characterized bacterial strains (Cordaillat-Simmons *et al*., 2020).

Within the European regulatory system, the monitoring of LBPs is done in collaboration between the European Medicines Agency (EMA) and the European Food Safety Authority (EFSA), depending on the nature of the claims. EMA regulates LBPs for therapeutic purposes as biologically active drugs and under the guidelines provided in the "Harmonized Monograph-European Pharmacopoeia Monograph 3053: Microbial Contaminants," which outlines the specifications for microbial purity, safety, and identity. For LBPs used as functional foods, EFSA regulates them, excluding those used in engineered microbes listed under the "Qualified Presumption of Safety" (QPS) list, and requires risk assessment for "Genetically Modified Microorganisms" (GMMs) in LBPs. These guidelines require the molecular description of genetic modification and the absence of transferable antibiotic resistance genes in LBPs (Cordaillat-Simmons *et al*., 2020).

There are important implications relating to the divergence between FDA and EMA/EFSA approaches to regulation, which have required highly flexible global development plans for pharmaceutical companies. For instance, the EMA regulatory approach to including LBP-derived products as biological medicinal products involves extensive molecular characterization and early-stage environmental assessment. Moreover, the two different approaches of EMA and EFSA entail suboptimal, fragmented approaches, so that it is possible to pursue early-stage clinical development in the United States, given the more integrated approach of the FDA. Omitted engineered strains of microorganisms need to be added to the EFSA QPS list; however, they demand extensive investment in risk assessments for GMMs. Moreover, both the FDA and EMA require Environmental Risk Assessment (ERA) for Engineered LBPs, which highlights the potential ecotoxicological risk impacts arising from strains released by patients as well as horizontal gene transfer. To promote global commercial development, increasing attention is being given to so-called "highest common denominator" approaches that entail sophisticated genetics-based biocontainment systems (such as "kill switches") and the absolute absence of any antibiotic resistance markers from inception to meet both the FDA's environmental concerns and the EMA's purity requirements simultaneously.

There is also an increasing recognition that such proactive harmonization of drug regulation is now considered crucial to gain access to venture capital investment in the quickly emerging area of synthetic biology (Group & Barberio, 2024; Cordaillat-Simmons *et al*., 2020). A summary of key safety challenges and biocontainment solutions in engineered probiotics is systematically summarized in Table 3.

4. Probiotics and NGPs in cancer therapy: From pathogenesis to inflammation modulation

Recent studies increasingly confirm the important role of the intestinal microbiota not only in maintaining health but also in cancer pathogenesis. Very recently, probiotics and next-generation probiotics, which have been defined as living microorganisms that exert beneficial health effects on their hosts, have been proposed as a novel anticancer agent (Thoda & Touraki, 2025).

Table 3. Summary of key safety challenges and biocontainment solutions in engineered probiotics

Safety Challenge	Technical Description	Engineered Solutions	Key Regulatory Considerations	References
HGT	Unintended transfer of therapeutic transgenes or antibiotic resistance markers from engineered strains to commensal or pathogenic microbiota.	Chromosomal integration of therapeutic genes instead of plasmid-based systems; implementation of toxin–antitoxin modules to enhance plasmid stability and limit gene spread.	Mandatory surveillance to confirm the absence of transferable antibiotic resistance genes and demonstration of genetic stability throughout clinical use.	Iacomino *et al*., 2024
Environmental Dissemination / Uncontrolled Survival	Persistence and proliferation of engineered microorganisms after treatment cessation or dissemination outside the host environment.	Biocontainment strategies based on synthetic auxotrophy, conditional metabolic dependencies, or CRISPR-Cas–based kill switches that induce self-destruction under non-permissive conditions.	Regulatory requirement for validated kill-switch systems and proof of environmental containment prior to approval.	Rottinghaus *et al*., 2020
Acquired Pathogenicity	Emergence or activation of virulence-associated traits as a consequence of genetic modification or host–microbe interactions.	Routine whole-genome sequencing and genetic surveillance; targeted deletion of known or predicted virulence factors from parental strains.	Strain-specific preclinical safety assessment and continuous post-engineering monitoring of pathogenic potential.	Iacomino *et al*., 2024

For years, inflammation has been known as an important part of the process of carcinogenesis and can be considered a risk factor for the development of various cancers, including Colorectal Cancer (CRC) (Marashi *et al*., 2024). A crucial role in the regulation of homeostasis has been observed for the gastrointestinal microbiota, which, owing to dysbiosis (an alteration of this microbial population), can cause chronic inflammation, possibly acting as a tumorigenic process (Wang *et al*., 2022). There has been sufficient evidence lately that suggests the use of probiotics can be considered an effective means for inhibiting the process of inflammation-driven cancers, which occur in the context of dysbiosis. Certain strains of probiotics, mainly *Lactobacillus* and *Bif*, which fall into the category of non-pathogenic bacteria, possess anticancer activities by acting on immunomodulatory pathways. These microbes can stimulate innate immune responses, thereby reducing the concentrations of pro-inflammatory cytokines like TNF-alpha and IL-6, and simultaneously increasing concentrations of anti-inflammatory cytokines like IL-10. This immunomodulatory effect mainly occurs by simultaneously inhibiting NF-κB signaling, which can act as a regulator for the process of cancer development and progression (Kvakova *et al*., 2022). In addition, these bacteria can help aid the production of SCFA butyrate, which can strengthen the mucosal barrier and cause apoptosis of cancerous cells (Wang *et al*., 2022; Thoda & Touraki, 2025). In the context of mouse models of CRC, the use of *B. longum* has been observed to produce a substantial decrease in the amount of tumors, which, in effect, provides evidence for this method acting as an innovative therapeutic target for the treatment of cancers (Marashi *et al*., 2024; Wang *et al*., 2022).

4.1. Nanobiotechnology and NGPs in colorectal cancer

Despite the potential of conventional probiotics, their effectiveness can be impeded by their lack of survival within the gastrointestinal tract and the uncontrolled delivery process. Advances have contributed to the recent development of nanobiotechnology-mediated probiotics and non-NGPs, which target these biological limitations. These technologies utilize particular synthetic biology approaches, such as the delivery of therapeutic cargo via the chromosome and genetic circuits with logic gates, for improved properties in target strains, especially the localized release of anti-proliferative compounds in the colorectum for CRC therapies (O'Toole *et al*., 2017).

At the same time, progress in nanotechnology allows for the encapsulation of probiotic bacteria in biocompatible nanomaterials (e.g., nanoparticles of alginate and chitosan or lipids).

These nanomaterials create a physical shield around the bacteria, protecting them from acidic and bile salt environments, as well as pH- or redox-mediated delivery in the tumor environment. In addition to live bacterial therapies (LBTs), there is increasing interest in the use of probiotic-derived extracellular vesicles (EVs). EVs are natural and non-viable carriers of bioactive molecules like proteins, nucleic acids, and peptidoglycans. These factors promote cross-talk between the host and microbes while preventing bacterial translocation in the human or animal barrier, which makes them particularly safe for immunocompromised persons. Briefly, *L. reuteri* EVs (REVs) have been found to have high stability in vivo and anti-tumorigenic effects through reconstitution of the tumor environment and its clearance (Kvakova *et al*., 2022).

The use of nanotechnology-mediated delivery systems for both NGPS (live strains) and probiotic EV (cell-free) products has widened the horizon of precision medicine (Liu *et al*., 2024; Wei *et al*., 2025). This represents a shift away from the conventional method of oral supplementation to more precise cancer therapeutic approaches. In this regard, approaches including nanotechnology-mediated delivery of live strains and the use of stable microbial vesicles, as discussed in Table 4, offer anticancer activities by various pharmacological actions.

Table 4. Probiotic, next-generation probiotic, and cell-free intervention strategies in cancer modulation

Probiotic / Intervention Type	Key Biomolecule / Payload	Targeted Cancer	Primary Mechanism	Evidence Level	Reference
Traditional Probiotics (e.g., *Lactobacillus*)	SCFAs; native cytokines (IL-10)	CRC	Anti-inflammatory modulation; TLR-mediated immune activation	Animal models (in vivo)	Ha *et al*. (2024); Wang *et al*. (2022)
Engineered Next-Generation Probiotics (Live Biotherapeutics)	Recombinant proteins (IL-12); logic-gate genetic circuits	CRC	Precision delivery of anti-proliferative payloads; site-specific therapeutic action	Preclinical (in vivo / in vitro)	O'Toole *et al*. (2017); Liu *et al*. (2024)
Probiotic-Derived Extracellular Vesicles (Cell-Free Derivatives)	REVs (proteins, RNA, peptidoglycans)	CRC	Tumor microenvironment (TME) modulation; restoration of microbial homeostasis via stable vesicles	In vitro and preclinical	Kvakova *et al*. (2022)
Nanotechnology-Enabled Probiotics	Encapsulated live strains (polymer-coated)	Gastrointestinal malignancies	Physical protection against gastric pH and bile; stimuli-responsive release	Preclinical models	Kim *et al*. (2023); Thoda & Touraki (2025)
Multi-Strain Probiotic Formulations	Synergistic metabolite profiles	Inflammation-associated cancers	Enhanced epithelial barrier integrity; broad-spectrum immune regulation	Animal models	Marashi *et al*. (2024)

5. Future directions: From diagnostic biosensors to personalized smart therapeutics

The path that designer probiotics will take will be dependent on integrating advances in synthetic biology with the general move toward personalized therapeutic approaches in healthcare.

Gene-editing tools, including CRISPR-Cas, are playing an increasingly pivotal role not only in therapeutic gene integration but also in the enhanced construction of safety control methods. Such control methods are believed to represent an essential technological achievement with respect to obtaining regulatory approval. One of the most promising areas of research involves the application of NGPs to the construction of real biosensors with living microorganisms. These microbes can detect and report the presence of biological markers for diseases inside the gut. For instance, there are probiotics that can identify biologically relevant signals such as tetrathionate and thiosulfate, or rather heme, related to inflammation or bleeding in the gut, and yield detectable signals such as pigments or fluorescence.

These diagnostic methods hold significant potential in the early diagnosis and treatment of gastrointestinal disorders and would help in the transfer of synthetic biology from the laboratory bench to the clinic (Abouelela & Helmy, 2024; Rottinghaus *et al*., 2020). The range of clinical applications considered for NGPs is quite extensive.

Proof-of-concept studies conducted at the preclinical and clinical stages have confirmed the efficacy of these microbes in treating IBD through IL-10 gene expression, treating symptoms associated with IBS, treating metabolic diseases, treating gastrointestinal malignancies, and bacterial infections caused by *Clostridioides difficile* and *Helicobacter pylori* (Pham *et al*., 2024; Wei *et al*., 2025). The application of multimodal delivery systems is imperative to improve clinical efficacy. The utilization of BEVs with biocompatibility to serve as nanocarriers for targeted therapy delivery represents one of the promising routes to enhance clinical efficacy. The BEVs can circumvent the difficulties involved in colonization and stability due to the live strain while preserving the advantages of the designed molecules (Liu *et al*., 2024; Wei *et al*., 2025).

The future of NGPs in terms of lasting success lies in the realm of personalized medicine and smart probiotics. The usual dietary advice does not take into account the individual differences in microbiota structure and reactivity to nutrients.

There is an acute need for personalized analyses of microbiota to suit the biological needs of each person. The next level of progress in this area is smart strains. These are microbes designed to work only if needed, in response to a prescribed signal (for instance, inflammation or antibiotics).

This is more sophisticated than the regular ingestion of vitamin supplements in terms of targeting the correct time for dosing, rather like precision medicine. Efficacy in such areas needs to be accompanied by parallel progress in methods of biological engineering and technologies related to microcapsules responding to external signals (Rottinghaus *et al*., 2020).

5.1. Next-generation probiotic engineering: Integration of artificial intelligence, multi-omics, and metabolic modeling

The field of probiotic engineering is at the cusp of a paradigm shift due to the integration of computational biology with microbiology. While conventional approaches to discovering probiotics relied exclusively on the principles of empirical screening and serendipitous discovery, current innovations in the field are slowly but steadily propelling the approach toward data-driven rational design.

This has become possible owing to the integration of three different but complementary technologies: artificial intelligence, multi-omics modeling, and metabolic network simulation (Tiwari *et al*., 2024; Wei *et al*., 2025).

AI technology has recently proven to be a critical tool that enhances the discovery and optimization of functional strains (Yu *et al*., 2025). Through the evaluation of vast biological databases, AI and machine learning models can forecast intricate interactions between hosts and microbes, which are challenging to elucidate using traditional scientific experiments (Probul *et al*., 2024). For example, AI-based systems can now be used to discover potential next-generation probiotics by linking specific genomic characteristics with favorable phenotypic attributes, thus ensuring efficiency from discovery to deployment (Probul *et al*., 2024).

Simultaneously, modeling based on multi-omics, including genomics, transcriptomics, proteomics, and metabolomics, provides an opportunity for holistic modeling of probiotic activity within the host milieu. When coupled with simulations of metabolic networks, it is possible to reconstruct models of bacterial metabolic pathways and prognosticate metabolite production based on different physiological scenarios.

Moreover, through this systems biology approach, not only can experimental designs be optimized before their validation in the wet lab, but also personalized therapies based on an individual's microbiome fingerprint can be developed (Marcos-Zambrano *et al*., 2023). Finally, through their convergence, the new standard of precision health is, in fact, bringing forth engineered probiotics based on their predictable safety and improved potency (Yu *et al*., 2025).

6. Conclusion

The advent of microbiome-targeting therapies also represents an evolution from broad-spectrum approaches intended for general gut health maintenance towards precision approaches. There is a clear therapeutic spectrum for both oncology and inflammation: conventional probiotics offer a basic but humble immunomodulatory role; NGPs offer deliberate precision through the controlled delivery of metabolites and immunotherapeutic payloads (e.g., short-chain fatty acids and IL-10) using genetic switches based on synthetic biology; and those that have emerged from this cell-free technology, like extracellular vesicles, provide an improved and non-replicating option that obviates the dangers of translocation of bacterial components. Nevertheless, in order to bring about the translation of these technologies

in the current scenario, it is necessary to adopt novel biocontainment methodologies, such as CRISPR/Cas kill switch technologies, which will become an important tool in addressing both concern mechanisms and safety in the biological environment. While this particular technology moves toward the development of diagnostic biosensors with the aim of applying personalized microbial therapies, the integration of synthetic biology tools in collaboration with nanotechnology-based delivery platforms has the potential to set the tone in cancer treatment as it stands today.

Conflict of interest

The authors declare no conflict of interest.

Ethical approval

This article does not contain any studies with human participants or animals performed by any of the authors.

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Authors' Contributions

Kimiya Khani-Chegeni (Data collection and analysis), Samaneh Sedighi-Khavidak (Supervisors of the project), Mohammad Rabbani Khorasgani (Advisor of the project) and Farnaz Mozayani (Participated in the research platforms). All authors have read and approved the final version of the manuscript.

Declaration of Artificial Intelligence

During the preparation of this manuscript, the authors used ChatGPT to improve the clarity and readability of the text. Additionally, Gemini (Google) was used to generate Figure 1. After using these tools, the authors thoroughly reviewed, revised, and edited the content and the visual representation to ensure accuracy and appropriateness. The authors take full responsibility for the integrity and originality of the manuscript's content, including the AI-assisted illustration.

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