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Exploring the potential of probiotic bacteria for drug delivery in humans

Ghosh Arunima and Dutta Sourav*

International Institute of Innovation and Technology, DH-6/24, Street No: 0317, Action Area 1D, Newtown, Kolkata, West Bengal - 700156, India

ABSTRACT

Probiotic bacteria represent a frontier in drug delivery, harnessing the natural colonization of the human gut to achieve targeted, efficient therapeutic release while mitigating systemic toxicities associated with conventional methods. This comprehensive review synthesizes over 30 studies on probiotic applications, focusing on the following key objectives: (1) screening diverse strains for drug delivery suitability, (2) optimizing gut delivery mechanisms under physiological conditions, (3) evaluating toxicity and safety profiles, and (4) assessing outcomes from animal studies, including side effects. The central themes encompass strain-specific attributes of *Lactobacillus* and *Bifidobacterium* genera, advanced techniques such as encapsulation, biofilm formation, and genetic engineering for controlled drug release, and their implications for treating cancers, neurological disorders, and gut dysbiosis. Evidence from preclinical models demonstrates enhanced viability (up to 90% post-transit), reduced tumor biomarkers, and anti-inflammatory effects, with low toxicity due to their commensal properties. However, challenges include maintaining strain viability, precise drug release control, and scalability for human trials. Thematic comparisons reveal that *Lactobacillus* strains outperform in acid tolerance and adhesion, while Bifidobacterium excels in immune modulation, although the evidence strength is moderate, primarily from in vitro and rodent models, with gaps in long-term human data and multi-strain interactions. The expected outcomes include revolutionary biocompatible delivery systems that improve bioavailability and patient convenience, potentially transforming precision medicine. This review identifies critical gaps, such as regulatory barriers and microbiome recovery post-exposure, and proposes future directions for clinical translation, emphasizing the need for rigorous human trials to validate safety and efficacy.

KEYWORDS: Probiotic drug delivery, *Lactobacillus* and *Bifidobacterium*, Targeted therapeutic delivery, Gut microbiome modulation, Encapsulation and biofilm systems, Live biotherapeutics

*Corresponding author:

Dr. Sourav Dutta

International Institute of Innovation and Technology

DH-6/24, Street No: 0317, Action Area 1D, Newtown, Kolkata**,** West Bengal – 700156, India

Email: sourav.dutta@i3tk.org, Mob No - 9681193038

INTRODUCTION

Drug delivery is a pivotal aspect of modern medicine, and researchers are continually seeking innovative strategies to administer therapeutics efficiently, effectively, and with minimal adverse effects.[1,2,3] Traditional approaches often grapple with challenges such as poor bioavailability, non-specific targeting leading to systemic toxicity, and suboptimal release kinetics, particularly for gastrointestinal (GI) applications.[4,5] For instance, many drugs degrade in the acidic stomach environment or fail to reach intended sites, such as the colon, resulting in reduced efficacy and increased dosing requirements.[6] In this context, probiotic bacteria—live microorganisms that confer health benefits when administered in adequate amounts—have emerged as promising vehicles for targeted delivery.[7,8] These commensal microbes, predominantly from genera such as *Lactobacillus* and *Bifidobacterium*, naturally inhabit the human gut and modulate the microbiome to support digestion, immune function, and pathogen resistance.[9] By leveraging their ability to survive GI transit, adhere to mucosal surfaces, and interact with host cells, probiotics can be engineered or encapsulated to carry payloads, such as anticancer agents, metabolites, or nucleic acids, directly to disease sites.[4,10]

The primary objective of exploring probiotic bacteria for drug delivery in humans is to assess their feasibility through laboratory experiments and animal studies, with the aim of developing safe, effective, and convenient systems that address conventional limitations.[11] The specific objectives are as follows: (1) screening probiotic strains to identify those with optimal properties for payload integration; (2) evaluating delivery efficacy in the gut under varying conditions and determining release optima; (3) investigating the toxicity of both probiotics and delivered drugs; and (4) conducting animal studies to gauge therapeutic outcomes and side effects.[4] Disruptions in the gut microbiome, linked to conditions such as colorectal cancer, inflammatory bowel disease, and neurological disorders via the gut-brain-immune axis, underscore the therapeutic potential of probiotics.[11,12] Studies have shown that these bacteria enhance nutrient absorption, lower cholesterol, and stimulate immunity, positioning them as biocompatible carriers superior to synthetic nanoparticles in terms of gut survival.[6] This review synthesizes evidence from diverse studies, highlighting probiotic innovations that could revolutionize drug administration, with expected outcomes of improved global health through targeted, low-toxicity therapies.

STRAIN SCREENING AND SELECTION

The screening of probiotic strains for drug delivery potential directly addresses the objective of identifying candidates with superior viability, adhesion, and modifiability, ensuring that they can withstand GI stress while effectively transporting payloads.[13,14] Screening protocols emphasize phenotypic and genotypic traits, including acid and bile tolerance, pathogen inhibition, non-hemolytic activity, and epithelial adhesion, often using multi-omics approaches such as 16S rRNA sequencing and real-time PCR for quantification.[15,16]*Lactobacillus* species, such as *L. gasseri*, *L. plantarum*, *L. casei*, *L. fermentum*, and *L. delbrueckii*, dominate the selection due to their prevalence in the human gut and ability to ferment oligosaccharides for colonization.[17,18] For example, *L. gasseri HG8* and *L. fermentum HG3* exhibited robust survival at pH 2.5 and 0.3% bile salts, strong inhibition of *Escherichia coli* and *Salmonella*, and high adhesion to epithelial cells, marking them as the top candidates. *L. plantarum* stands out for biofilm formation and nanoparticle synthesis, enhancing delivery stability.[19,20]

Bifidobacterium species, including *B. bifidum*, *B. infantis*, and *B. longum*, are similarly screened for their immunomodulatory roles and culturability, although viability challenges persist; real-time PCR revealed only 2% culturability in some formulations, prompting strain optimization.[15,21] Next-generation probiotics, such as *Akkermansia muciniphila* and *Faecalibacterium prausnitzii*, are gaining traction for dysbiosis reversal in metabolic and neuropsychiatric diseases, with culturomics enabling their isolation and multi-omics characterizing their health benefits.[16] Comparative assessments have shown that *Lactobacillus* outperforms in antibiotic tolerance but lags in engraftment diversity compared to *Bifidobacterium*, which better modulates inflammation.[12,22] Trade-offs include variable culturability (e.g., 62% for *Lactobacillus*) and anaerobic cultivation hurdles for *Akkermansia*, necessitating tailored screening.[15,16] The strength of evidence is high for in vitro traits but moderate for in vivo translation, with gaps in strain-specific responses to non-antibiotic drugs.[22]

Table 1.Comparison of Probiotic Strains for Drug Delivery Potential

Strain	Key Attributes	Limitations	References
Lactobacillus gasseri HG8	High acid/bile tolerance (pH 2.5, 0.3% bile), pathogen inhibition (E. coli, Salmonella), epithelial adhesion	Variable culturability (62%)	[15]
Lactobacillus plantarum	Biofilm formation, selenium nanoparticle synthesis, oligosaccharide fermentation	Sensitivity to some non-antibiotics	[19,20]
Bifidobacterium infantis	Immune modulation, gut colonization, diversity enhancement	Low viability in storage (2%), anaerobic needs	[15,21]
Akkermansia muciniphila	Dysbiosis reversal, metabolic benefits (e.g., obesity), mucin degradation	Anaerobic cultivation challenges, low abundance	[16]
Faecalibacterium prausnitzii	Anti-inflammatory effects, butyrate production for gut barrier	Difficult cultivation, oxygen sensitivity	[8]
Lactobacillus casei	Tumor targeting, SeNP encapsulation, biomarker modulation	Moderate bile tolerance	[20]

This process ensures that selected strains, such as *L. gasseri* and *B. infantis*, are primed for engineering, with *Lactobacillus* favored for cancer applications because of its versatility.[10]

OPTIMAL GUT DELIVERY CONDITIONS AND MECHANISMS

Optimizing probiotic drug delivery to the gut fulfills the objective of evaluating strain capabilities under physiological conditions, focusing on encapsulation, biofilms, and genetic engineering to achieve controlled release.[4] Gut conditions—pH gradients (2-8), bile salts (0.3-2%), and peristalsis—demand robust protection; optimal delivery targets the small intestine for immune activation near Peyer's patches or the colon for microbiome modulation.[6,23] Encapsulation using biopolymers such as polysaccharides or alginate shields probiotics, with microencapsulation via extrusion, emulsion, or

spray-drying yielding 70-90% viability post-storage and targeted release.[5,6,24] Layer-by-layer (LbL) assembly further enhances adhesion and growth, protects against GI insults, and enables in vivo enrichment.[25] For instance, LbL-encapsulated strains survived gastric simulation better than free cells, releasing payloads at neutral pH.[25]

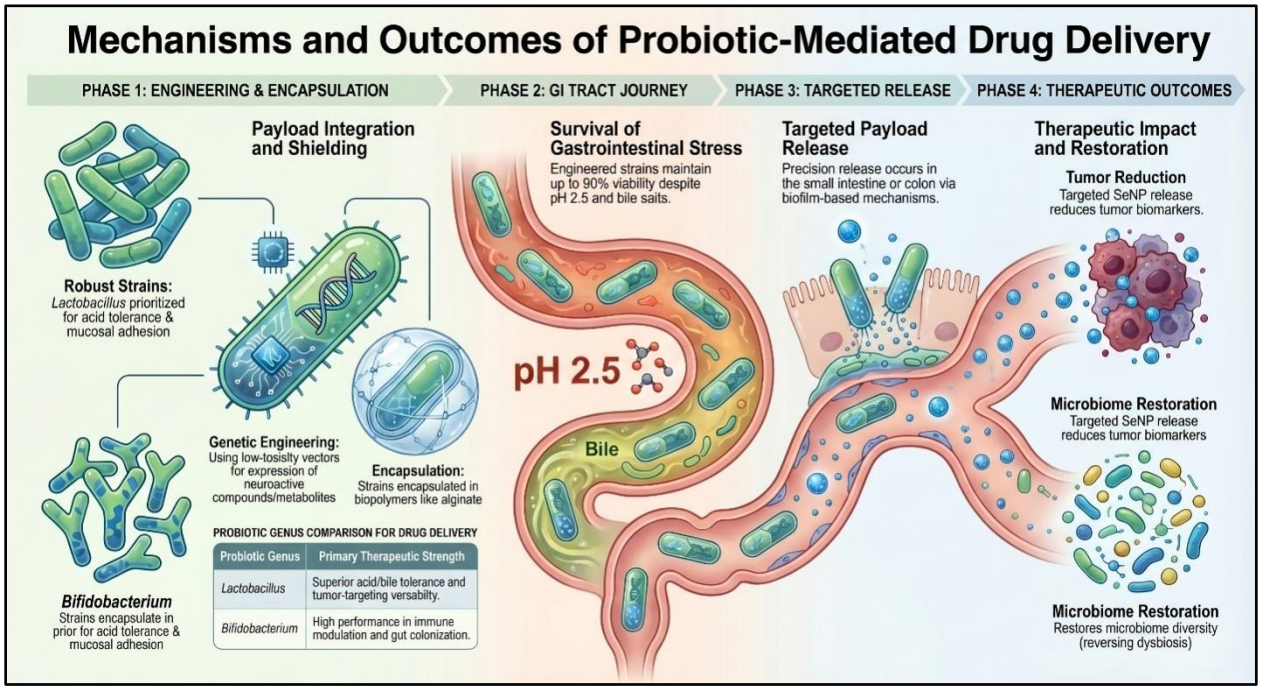

Figure 1 Conceptual Framework for Probiotic-Mediated Drug Delivery in Humans

The delivery process begins with the screening and selection of robust strains, primarily from the *Lactobacillus* and *Bifidobacterium* genera, characterized by high acid and bile tolerance. To ensure viability during gastrointestinal (GI) transit, these strains undergo optimization via genetic engineering for payload expression or encapsulation using biopolymers like alginate and layer-by-layer (LbL) assembly. Encapsulation provides 70-90% survival post-transit, shielding the bacteria from the harsh environment of the stomach (pH 2.5). Upon reaching the target site (e.g., the small intestine or colon), the probiotics adhere to mucosal surfaces and release therapeutic payloads, such as biogenic selenium nanoparticles (SeNPs) or neuroactive compounds. Preclinical outcomes demonstrate that this targeted approach can effectively reduce tumor biomarkers, modulate the gut-brain-immune axis, and reverse gut dysbiosis with minimal systemic toxicity compared to traditional therapies.

Biofilm-based mechanisms leverage self-produced or food-grade matrices for stability, improving tolerance to stress, and allowing incorporation into foods; biofilms on surfaces such as cellulose promote proliferation and specific gut enrichment.[19] Genetic engineering introduces circuits for payload expression, such as nucleic acids or metabolites, using low-toxicity vectors, such as attenuated *Lactobacillus*, homing to tumors or inflamed sites.[4,26] In neurological contexts, engineered probiotics deliver neuroactive compounds via the gut-brain axis, optimizing metabolite production, such as vitamin B12.[11,27] Cellulose hydrogel bioreactors simulate gut gradients, supporting multi-strain cultures and scalable production.[28] Optimal conditions include prebiotic supplementation (e.g., oligosaccharides) to boost engraftment, with trade-offs in release kinetics; biofilms excel in colonization but may delay immediate release compared to engineered strains.[19,24] Evidence from in vitro models has shown superior performance of selenium nanoparticle delivery for colon targeting.[20] Gaps include strain-specific optimization for diverse gut microbiomes, with moderate evidence strength from simulation studies.[6]

SAFETY AND TOXICITY PROFILES

Assessing the toxicity of probiotic bacteria and their drug payloads addresses the objective of ensuring safety, revealing generally low risks due to their commensal status and biocompatibility.[4,10] Probiotics such as *Lactobacillus* and *Bifidobacterium* demonstrate non-hemolytic profiles, antibiotic resistance without promoting spread, and anti-inflammatory effects, minimizing systemic impacts.[12] Non-antibiotic drugs inhibit 24% of gut strains in vitro; however, recovery occurs via community adaptation, with transient dysbiosis as the primary side effect.[22,29] In cancer models, biogenic SeNPs from *L. plantarum* and *L. casei* modulated p53 and TNF-α without toxicity, outperforming inorganic forms in terms of bioavailability.[20]Bifidobacterium strains in neonatal studies safely reversed dysbiosis, increasing diversity without adverse events.[21]

Toxicity profiles vary by strain; *Lactobacillus* shows resilience to antipsychotics, whereas Bifidobacterium may experience shifts in methane metabolism pathways.[12,22] Compared to viral vectors, probiotics induce milder immune responses, although rare activations, such as monocyte inflammation, can occur.[10] Safety evidence is strong from short-term studies, but gaps exist in long-term exposure, especially for engineered strains.[29] Overall, these profiles support human applicability and have implications for reducing drug-induced microbiome disruptions.[11]

ANIMAL STUDY OUTCOMES AND SIDE EFFECTS

Animal studies evaluating probiotic drug delivery efficacy and side effects align with the objective of preclinical validation, demonstrating targeted benefits in disease models with minimal adverse outcomes.[4] In rat colorectal cancer induced by 3-MBA, *L. casei*-derived selenium nanoparticles reduced EGFR, MMP-9, and oxidative stress, improving histopathology over two weeks without toxicity.[20] Murine multiple sclerosis models showed that *Lactobacillus* and *Bifidobacterium* decreased inflammatory monocytes and HLA expression, enriched beneficial taxa, and modulated the gut-brain-immune axis.[11,12] Neonatal rat simulations with B. bifidum and *L. acidophilus* reversed dysbiosis, boosted diversity, and reduced Clostridium, with rapid recovery from minor shifts.[21]

Feedlot cattle trials have confirmed microbiome modulation, enriching nutrient pathways without side effects.[30] Engineered vectors enhance tumor homing and immunity in vivo, with low engraftment variability as the main trade-off.[26] Side effects were limited to transient dysbiosis, resolving within months, in contrast to the higher toxicities of traditional therapies.[29] The strength of the evidence is promising but preclinical-focused, with gaps in chronic dosing.[10]

Table 2. Outcomes of Preclinical Studies on Probiotic Drug Delivery

Model/Disease	Probiotic/Method	Key Efficacy Outcomes	Side Effects/Noted Trade-offs	References
Colorectal Cancer (Rat)	*L. casei* SeNPs (Encapsulation)	Reduced tumor biomarkers (EGFR, MMP-9), antioxidant boost, histopathological improvement	None reported; short-term dosing	[20]
Multiple Sclerosis (Murine)	*Lactobacillus/Bifidobacterium* (Oral, Genetic)	Decreased inflammation, microbiome enrichment (*Lactobacillus* increase), HLA modulation	Transient dysbiosis; variable engraftment	[11,12]

Gut Dysbiosis (Neonatal Rat)	*B. bifidum/L. acidophilus* (Dual-strain)	Increased beta diversity, pathogen (Clostridium) reduction	Minimal; rapid recovery within weeks	[21]
Tumor Therapy (In vivo Mouse)	Engineered Attenuated Bacteria (Vectors, Biofilm)	Enhanced immunity, tumor homing, metabolite delivery	Low toxicity; engraftment variability	[4,19,26]
Metabolic Dysbiosis (Cattle)	Lactic Acid Bacteria (Multi-strain)	Enriched digestion pathways, health benefits	None; period-dependent shifts	[30]

CHALLENGES

Despite promising advancements, challenges in probiotic drug delivery include maintaining bacterial viability during processing and GI transit, controlling precise drug release, and ensuring scalability.[4,6] Viability drops in harsh conditions (e.g., 2-62% culturability), necessitating advanced encapsulation, although methods such as spray-drying trade efficiency for cost.[15,24] Drug release control is hindered by variable gut pH and microbiome interactions, with non-antibiotics affecting 24% of strains and promoting resistance risks.[22,29] Scalability issues arise in genetic engineering and biofilm production, compounded by regulatory gaps in live biotherapeutics.[10,19] Multi-strain dynamics and long-term engraftment remain underexplored, with evidence highlighting the need for standardized assays.[16] These hurdles underscore the gaps in translating preclinical successes, particularly in diverse populations.[11]

DISCUSSION

Synthesizing themes from screened strains, optimized mechanisms, safety profiles, and animal outcomes, probiotic bacteria provide a versatile, targeted platform for drug delivery, with *Lactobacillus* and *Bifidobacterium* being central to gut applications.[4] Encapsulation and biofilms enhance viability over free cells (70-90% survival), while genetic engineering enables precision in cancer and neurological therapies, outperforming traditional methods in biocompatibility.[11,19,25] Safety is robust, with low toxicity and rapid microbiome recovery, although non-antibiotic interactions reveal

vulnerabilities.[22,29] Preclinical efficacy, including tumor reduction and dysbiosis reversal, is evident, but trade-offs such as engraftment variability persist.[20,21]

Thematically, *Lactobacillus* excels in adhesion and nanoparticle delivery for oncology, while *Bifidobacterium* aids immune modulation in neurology, with moderate evidence strength from animal models (e.g., reduced inflammation in MS) but gaps in human trials, multi-omics integration, and regulatory frameworks.[10,12,16] Gap analysis points to underexplored long-term effects, strain synergies, and scalability, limiting translation.[6] The implications for medicine include enhanced bioavailability for precision therapies, reduced antibiotic reliance, and improved outcomes in dysbiosis-linked diseases.[26] The expected outcomes of this exploration include safe and innovative systems that revolutionize drug delivery and foster global health advancements through convenient and effective interventions.[4]

CONCLUSION

In conclusion, this review illuminates the transformative potential of probiotic bacteria as drug delivery vehicles in humans, integrating insights from strain screening, mechanism optimization, safety evaluations, and animal studies to propose a paradigm shift in therapeutics. By addressing key objectives, such as identifying robust strains like *Lactobacillus* and *Bifidobacterium*, refining gut delivery via encapsulation and engineering, confirming low toxicity, and validating efficacy in preclinical models, this approach offers safe, effective, and convenient alternatives to conventional methods.[4,10] The expected outcomes encompass the development of biocompatible systems that enhance bioavailability, target diseases such as cancer and neurological disorders, and mitigate side effects, potentially revolutionizing medicine and improving health worldwide. Continued research bridging the gaps to human trials will realize these benefits, contributing significantly to global well-being through innovative microbiome-informed strategies.

Implications for Human Translation and Future Directions for Human Trials

Probiotic drug delivery holds substantial translational promise, with animal successes in tumor targeting and microbiome modulation indicating its viability for human applications.[4,20] Engineered strains could amplify immunotherapy and reduce systemic toxicities; however, human trials are essential to confirm their safety, efficacy, and microbiome stability.[10,11] Future directions include phase I trials assessing viability in diverse cohorts, multi-strain formulations for synergistic effects, and longitudinal

studies on resistance.[16,22] Regulatory progress and scalable manufacturing, informed by gaps in current evidence, will integrate these into precision medicine, offering personalized treatments for gut and systemic conditions.[29]

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