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Biotechnological Approaches to Probiotics and Postbiotics through the Gut-Brain Axis Modulation

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ABSTRACT

The gut microbiome and gut-brain axis are central to systemic homeostasis, with dysbiosis implicated in neurodegenerative (Alzheimer's and Parkinson's) disorders and gastrointestinal conditions (inflammatory bowel disease, irritable bowel syndrome, and fibromyalgia). These conditions are marked by a reduction in beneficial taxa (e.g., *Faecalibacterium prausnitzii* and Bifidobacterium) and an increase in harmful ones (e.g., *Escherichia coli* and *Clostridium scindens*). Probiotic, prebiotic, postbiotic, and symbiotic interventions show therapeutic promise, but results can vary. Biotechnological advances, including CRISPR-Cas9 and genetic kill-switch systems, enable precision-engineered probiotics and postbiotics, though concerns about safety, genetic stability, gene transfer, and immune compatibility persist. Encapsulation and nanoencapsulation strategies improve microbial viability and site-specific delivery; i.e., microencapsulating *Lactobacillus acidophilus* and *Bifidobacterium animalis* in alginate-pectin matrices enhances survival during freeze-drying, storage, and gastrointestinal transit. Advancing clinical translation requires integrating multi-omics and machine learning with bioengineering approaches. This review examines the microbial-neural interface and surveys emerging biotechnological strategies for improving microbial-based therapeutics. ***DOI: 10.22034/IBJ.30.4.193***

Keywords: Brain-Gut Axis, Dysbiosis, Gastrointestinal microbiome, Prebiotics, Probiotics

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1. INTRODUCTION

The gut-brain axis (GBA) is a two-way communication network linking the gastrointestinal (GI) tract and the central nervous system (CNS). This system integrates neural, endocrine, immune, and microbial pathways to regulate both physiological and pathological processes. The vagus nerve is the primary neural link, regulating appetite and metabolism,

especially in conditions like obesity and diabetes. In addition, the enteric nervous system, often referred to as the "second brain", operates semi-autonomously while maintaining direct interactions with the CNS, affecting emotional and stress-related behaviors. Hormonal signaling further reinforces GBA connectivity. For example, glucagon-like peptide-1 (GLP-1) serves as a metabolic regulator and facilitates communication between gut microbes and brain function[1]. Serotonin, predominantly synthesized in the gut, is another key signaling molecule involved in mood regulation and GI motility[2]. Furthermore, endocrine stress responses are coordinated via the hypothalamic-pituitary-adrenal (HPA) axis, which releases cytokines and chemokines that modulate immune activity in response to the microbial signal[3].

The gut microbiota contains approximately 10^{14} bacteria[4] and has been shown to exert significant influence on neurological health. Microbial imbalances have been implicated in the pathophysiology of disorders such as Parkinson's (PD) and Alzheimer's (AD). The gut microbiome, through its interaction with the GBA, plays a critical role in maintaining systemic homeostasis. Dysbiosis has been increasingly associated with chronic GI and neurological disorders, including inflammatory bowel disease (IBD), irritable bowel syndrome, AD, PD, and fibromyalgia. These conditions are often characterized by reduced beneficial microorganisms, such as *Faecalibacterium prausnitzii* and *Bifidobacterium* spp., alongside an increased abundance of potentially harmful bacteria, including *Escherichia coli* and *Clostridium scindens*. Microbiome-targeted interventions, including probiotics, prebiotics, postbiotics, and synbiotics, show therapeutic potential, although clinical outcomes remain inconsistent.

Emerging biotechnologies, including CRISPR-Cas9, genetic kill switches, encapsulation, and nano-encapsulation, may enable safer and more precise microbial therapies. However, challenges involving genetic stability, horizontal gene transfer, safety, and host immune compatibility remain. This review explores the microbial-neural interface and highlights how bioengineering, multi-omics, and machine learning (ML) may advance the efficacy and translation of microbiome-based therapeutics) disease, in part through their effects on microglial activation, neuro-inflammation, and synaptic plasticity[5]. Gut microbiota-derived metabolites, such as short-chain fatty acids (SCFAs), neurotransmitters, and neuromodulators, can traverse the blood-brain barrier, modulate neuro-transmitter systems, and influence CNS integrity. These interactions underscore the therapeutic potential of microbiota-based interventions[5]. Dysbiosis has been linked to altered brain connectivity and accelerated aging in conditions, highlighting the role of the microbiota in neuropsychiatric health[6].

The GBA can be modulated through interventions involving microbiota, probiotics, and postbiotics. Probiotics are live microorganisms that, when administered in adequate amounts, confer a health benefit on the host and promote gut health by enhancing the intestinal barrier, modulating immunity, and suppressing harmful bacteria. In contrast, postbiotics are non-living microbes or their components that offer health benefits through bioactive compounds like SCFAs, exopolysaccharides, and bacteriocins. Postbiotics are more stable and safer than probiotics, which avoid the risks linked to live microorganisms[7].

Engineering the gut microbiome has emerged as a powerful strategy for modulating host physiology and influencing the GBA, positioning probiotics as programmable living therapeutics. Advances in metagenomics, ML, and genetic engineering have improved the identification and optimization of microbial functions related to neurological, metabolic, and immune regulation. Metagenomic profiling enables the discovery of microbial strains and gene signatures associated with homeostasis and disease[8]. Moreover, ML frameworks, ranging from adaptive regression models to deep-learning architectures, facilitate the prediction of metabolite outputs and the rational design of probiotic consortia capable of producing targeted neuroactive or immunomodulatory compounds[9]. Also, synthetic biology provides robust molecular tools; for instance, *E. coli* Nissle 1917 (EcN) can be engineered with synthetic promoters, genome-integrated circuits, and minimized plasmid systems to improve stability, tune expression levels, and reduce biosafety risks. CRISPR-Cas-enabled editing expands these capabilities by the deletion of harmful genes, insertion of therapeutic pathways, and targeted elimination of antibiotic-resistant pathogens. Engineered probiotics have been designed to manipulate metabolic pathways such as glucose dehydrogenase expression to disrupt cancer cell energetics or secrete anti-inflammatory or neuroactive molecules relevant to GBA modulation[10]. However, the translation of these strains into in vivo applications introduces significant containment challenges. Even sophisticated kill-switch systems remain susceptible to evolutionary escape under gut-like conditions, necessitating multilayer biocontainment strategies. Collectively, these developments show both the transformative potential of engineered microbiomes and the biological constraints that must be overcome to achieve safe and reliable next-generation microbial therapeutics[11].

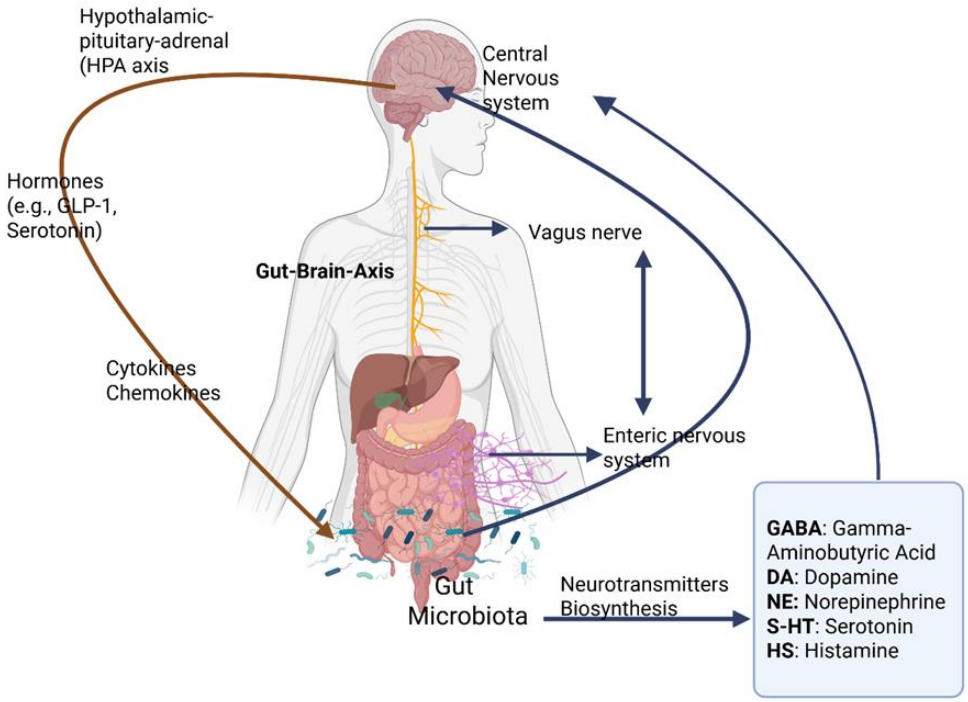

Fig. 1. Schematic representation of bidirectional communication within the GBA, illustrating neural (vagus nerve), endocrine (HPA axis and gut hormones), and immune signaling pathways linking gut microbiota to the CNS. Microbial metabolites and neurotransmitters (e.g., GABA, dopamine, serotonin, and histamine) modulate neural, metabolic, and behavioral functions (created by BioRender.com).

1.1. GBA: microbial diversity and composition

The gut microbiota is the largest microbial environment in the human body, containing more than 10^{13} to 10^{14} bacteria. Collectively, these microorganisms possess a genetic repertoire—referred to as the microbiome—which is estimated to be ten times larger than the human genome. Recent estimates suggest that the ratio of microbial and human cells in the body approaches 1:1[12]. This microbial community develops rapidly after birth and typically reaches structural and functional maturity between the ages of two and three. The adult gut is home to roughly 500 to 1,000 distinct bacterial species, with the dominant phyla being Firmicutes, Bacteroidetes, Actinobacteria, and Proteobacteria. These microbes exist in a mutualistic or commensal relationship with the host and play essential roles in maintaining physiological equilibrium. A decline in microbial diversity or a shift in composition—known as dysbiosis—can disrupt host-microbe interactions, alter metabolic outputs, and contribute to various diseases[13,14]. Microbial metabolites are bioactive compounds produced by gut microbiota through the fermentation of dietary components. These metabolites play crucial roles in host metabolism, immune regulation, and maintaining GBA homeostasis.

1.1.1. Mechanisms of SCFA action

SCFAs, primarily acetate, propionate, and butyrate, are produced through the fermentation of dietary fiber by the gut microbiota. These compounds help preserve the integrity of the intestinal barrier, enhance mucosal secretion, and exert anti-inflammatory effects. SCFAs exert their functions by activating G protein-coupled receptors (GPCRs), including GPR41, GPR43, GPR109A, and GPR164, on epithelial and immune cells. This receptor signaling modulates the release of hormones such as GLP-1 and peptide YY, as well as insulin secretion from pancreatic β-cells, ultimately influencing appetite and glucose metabolism[15,16]. Butyrate and propionate also exhibit anti-inflammatory properties by reducing the expression of cytokines and nitric oxide via GPCR signaling, thus contributing to immune homeostasis[17,18]. In addition, acetate and butyrate play roles in lipid metabolism by converting to acetyl-CoA, which serves as a substrate for energy production and triglyceride synthesis. These SCFAs can also activate AMP-activated protein kinase, thereby upregulating PGC-1α and modulating transcription factors like PPARα and PPARγ that govern lipid utilization. Furthermore, SCFAs influence lipolysis through hormone-sensitive lipase and adipose triglyceride lipase, thereby modulating fat storage and mobilization[19,20].

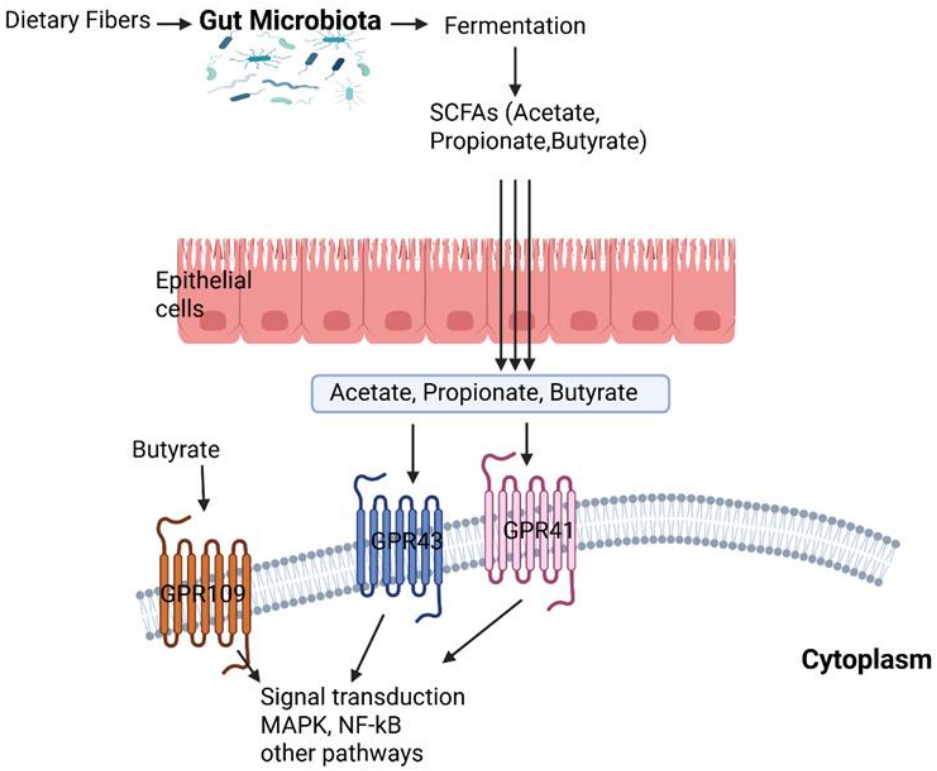

Fig. 2. Gut microbial metabolites showing the functional effect of SCFAs on signal transduction pathways (created by BioRender.com).

1.1.2. Neurotransmitter production

Emerging evidence indicates that the gut microbiota significantly influences the biosynthesis of key neurotransmitters, including γ-aminobutyric acid (GABA), dopamine, norepinephrine, serotonin, and histamine. These microbial activities affect both central and enteric nervous system functions and are linked to mood regulation and neuropsychiatric conditions. Neurotransmitters can be broadly categorized into amino acid derivatives (e.g., glutamate [Glu], GABA, and glycine), monoamines (e.g., dopamine and norepinephrine), trace amines, peptides, purines, and acetylcholine, all playing diverse roles in neural communication[21]. Glu is the principal excitatory neurotransmitter in the brain, crucial for learning, memory, and synaptic plasticity. It acts on ionotropic receptors, triggering calcium influx and downstream signaling pathways. GABA, derived from Glu, serves as the main inhibitory neurotransmitter, maintaining neuronal balance. Certain *Lactobacillus* species can enhance GABA synthesis and the expression of its receptors, thereby influencing anxiety and mood disorders. Glycine functions both as an excitatory and inhibitory neurotransmitter and acts in conjunction with Glu at NMDA (N-Methyl-D-Aspartate) receptors to support motor control and cognitive processing. Dopamine, synthesized in regions such as the substantia nigra and ventral tegmental area, regulates reward pathways, motor control, and emotional responses. It is produced from the amino acid tyrosine and can be further converted into norepinephrine (NE) and epinephrine. Gut bacteria, such as *Bacillus* and *Serratia*, are known to produce dopamine, which may interfere with the efficacy of levodopa (Levodopa or L-DOPA), a medication used for the treatment of PD. NE, in addition to its role in alertness and inflammation, has been shown to influence gut microbial composition. Notably, colonization of germ-free animals has been demonstrated to restore NE levels. Serotonin, largely synthesized in the GI tract, plays a central role in mood, digestion, and immune modulation. Its production is closely regulated by gut microbiota. Lastly, histamine, produced by gut bacteria like *Lactobacillus* and *Klebsiella*, influences arousal, appetite, and physiological homeostasis[22]. Aromatic amino acids, such as phenylalanine, tyrosine, and tryptophan, serve as microbial precursors for a variety of neuroactive compounds. Phenylalanine is a substrate for the synthesis of dopamine, norepinephrine, epinephrine, Glu, aspartate, and glycine. The metabolism of tryptophan results in the production of serotonin, niacin (vitamin B3), $NAD(P)^+$, and kynurenine pathway

metabolites like indole, which are implicated in immune regulation and psychiatric disorders, including depression and schizophrenia[23,24]. Bacterial genera such as *Escherichia*, *Lactobacillus*, and *Bifidobacterium* are actively involved in aromatic amino acid metabolism[25]. Trimethylamine N-oxide (TMAO), another gut-derived metabolite, has been linked to systemic and neuroinflammation, particularly in the aging population. TMAO forms when gut microbes convert dietary nutrients such as choline and L-carnitine into trimethylamine, which is then oxidized in the liver by flavin-containing monooxygenase 3. Elevated TMAO levels have been associated with inflammatory conditions and cardiovascular risks[26]. Furthermore, studies have shown that the exposure of human astrocytes to TMAO results in morphological changes and increased expression of activation markers, suggesting a direct role in astrocyte-mediated neuroinflammation. These findings underscore the potential of TMAO as a biomarker and therapeutic target for age-related cognitive decline[27].

1.2. Probiotics

Although traditional probiotics are widely regarded as beneficial for health, their clinical effectiveness and underlying mechanisms are often not fully defined[28-30]. Advances in high-throughput sequencing have uncovered gut microbes that are strongly associated with health, enabling the development of next-generation probiotics (NGPs)[31-34]. Also, using synthetic biology and bioinformatics, engineered probiotics can be tailored to target specific disease conditions, offering improved stability and viability[35].

1.2.1. Mechanisms of intestinal barrier function and regulation of neuroinflammation

The GI tract functions as a selectively permeable barrier, allowing the absorption of essential nutrients while preventing the entry of harmful pathogens and toxins. When this barrier becomes compromised—commonly referred to as "leaky gut"—it allows the translocation of microbial products and antigens, which can contribute to conditions such as IBD. The integrity of the intestinal barrier is primarily maintained by tight junction proteins (TJPs), including occludin, claudins, and zonula occludens, which are regulated by both host and microbial factors[36]. Probiotic strains such as *L. rhamnosus* LR 32, *B. lactis* BL 04, and *B. longum* BB 536 have been shown to strengthen the gut barrier by modulating the expression of TJPs. Additionally, *Lactobacillus* and *Bifidobacterium* species promote gut health by reducing inflammation, preventing pathogenic adhesion, producing bioactive compounds, and stimulating digestive enzymes, thereby supporting both GI and systemic health[37]. In contrast, pathogenic bacteria like *Salmonella typhimurium* and *E. coli* can disrupt the epithelial barrier, worsening inflammation and tissue damage[38]. The gut microbiota contributes to CNS regulation through immunological pathways and neurotransmitter modulation. For instance, *B. longum* CCFM1077 has been shown to reduce microglial activation in the cerebellum, influence neuro-transmitters such as glutamic acid and GABA, and improve kynurenine pathway metabolism by lowering quinolinic acid levels[39]. Probiotic strains such as *B. lactis*, *L. rhamnosus*, and *L. reuteri* have also demonstrated potential in modulating the GBA by reducing neuroinflammatory cytokines like interleukin-1β (IL-1β) and IL-6 in both the brain and GI tract. These effects help preserve intestinal integrity, decrease oxidative stress, and maintain homeostasis, thereby mitigating symptoms of anxiety and supporting overall brain health[40].

1.2.2. Pathophysiological context of gut-brain dysregulation

Dysbiosis has been linked to various neurological and inflammatory disorders. In PD, alterations in gut microbiota composition can affect neurotransmitter production and promote inflammation, contributing to disease progression. Similarly, in AD, reduction in beneficial phyla such as *Actinobacteria* and *Firmicutes* is associated with increased gut permeability and systemic inflammation[41]. In multiple sclerosis, gut dysbiosis characterized by elevated levels of *Bacteroides*, *Prevotella*, and *Streptococcus thermophilus* has been linked to immune dysregulation and disease advancement. Decreased populations of butyrate-producing Firmicutes and compromised intestinal integrity may also impair the blood-brain barrier[42]. Major depressive disorder and generalized anxiety disorder frequently co-occur with chronic conditions such as fibromyalgia and autoimmune diseases, amplifying symptoms and disability. These psychiatric disorders are associated with dysregulation of the HPA axis and are influenced by microbial endotoxins and inflammatory cytokines like IL-6 and tumor necrosis factor-alpha. Alterations in gut microbiota—particularly increased *Bacteroidetes* and *Proteobacteria* and decreased *Firmicutes*—have been reported in individuals with major depressive disorder[43]. Serotonin imbalance, significantly affected by microbial activity in the gut, has been implicated in mood disorders including depression. Decreased production of SCFAs, especially butyrate, which possesses antidepressant properties, may exacerbate depressive symptoms. Furthermore, gut-derived inflammation and HPA axis abnormalities further

Table 1. Comparative overview of traditional probiotics, NGPs, and engineered probiotics: mechanisms, stability, safety, regulatory pathways, and therapeutic potentials

Parameter	Traditional probiotics[28-30]	NGPs[28,31-34]	Engineered probiotics[35]
Typical organisms/origin	Mostly *Lactobacillus*, *Bifidobacterium*, etc., with long food-associated or historical human use	Often newly prioritized gut commensals linked to health via microbiome studies (broader taxa; many strict anaerobes)	Uses a chosen chassis strain (e.g., EcN, LAB) that is genetically modified to add new functions
Development logic	Benefit-by-consumption approach; strain selection often based on tradition + survivability + supportive trials	Function-first, selection based on mechanistic roles and association with disease/health; often positioned closer to biotherapeutics than foods	Design-first, rational engineering of circuits + payloads (enzymes, cytokines, nanobodies, sensing modules)
Mechanism	Broad and strain-dependent: competitive exclusion, barrier support, immune modulation, metabolite-mediated effects	More targeted and mechanistically defined (e.g., specific metabolite production, mucin utilization, immune-regulatory functions)	Programmable mechanisms: sense-and-respond logic, localized production/delivery of a defined therapeutic output; can also act as diagnostics
Stability/manufacturability	Scalable, but performance depends on viability, strain identity, and formulation/storage conditions	Many candidates are oxygen-sensitive/strict anaerobes, creating formulation and scale-up hurdles; oxygen-adaptation/synergy strategies are emerging	Requires drug-like CMC (Chemistry, Manufacturing, and Controls); additional concerns include genetic stability, expression burden, and containment design
Safety profile	Generally safe in healthy populations; adverse events are uncommon but risk rises in vulnerable groups; product quality matters	Often less historical safety exposure; safety evaluation is more case-by-case (novel taxa/strain-s pecific risks)	Adds GMO-specific risks: off-target host effects of payloads, shedding, horizontal gene transfer, circuit/kill-switch failure - typically assessed under drug-level paradigms
Regulatory positioning	Usually foods/supplements for wellness claims; becomes “drug-like” when intended for disease treatment/prevention	Frequently discussed as bridging toward live biotherapeutics when targeting disease endpoints (classification driven by intended use)	Commonly pursued as live biotherapeutic products/biological drugs (IND/CTA + GMP + environmental/GMO considerations depending on jurisdiction)
Evidence base/therapeutic role	Large clinical literature, but outcomes are heterogeneous across strains/products and endpoints	Growing evidence; translational bottlenecks (cultivation/formulation) and patient stratification remain key issues	Strong preclinical pipeline and expanding clinical activity; review tables summarize candidates in trials across indications

contribute to emotional disturbances[44]. While the exact pathogenesis of IBD remains under investigation, strong evidence supports a role for the gut microbiota. Individuals with IBD commonly exhibit reduced microbial diversity, lower levels of beneficial phyla such as *Bacteroidetes* and *Firmicutes*, and increased levels of *Proteobacteria*, notably *E. coli*. Commensal species like *F. prausnitzii* and *Roseburia hominis* are frequently diminished[45]. Fibromyalgia, which often coexists with irritable bowel syndrome and rheumatoid arthritis, presents with chronic pain, fatigue, cognitive impairments, and mood disorders. Low-grade systemic inflammation characterized by elevated levels of IL-6 and IL-8 has been implicated in increased pain sensitivity. Dysbiosis, including small intestinal bacterial overgrowth and a decline in butyrate-

producing bacteria such as *F. prausnitzii*, may aggravate inflammatory responses and GI symptoms[46,47]. Notably, both irritable bowel syndrome and fibromyalgia are marked by reductions in the abundance of *Bifidobacterium* and *Ruminococcaceae* (including *F. prausnitzii*), alongside increased levels of *C. scindens*[48].

1.2.3. Gut microbiome modulation, challenges and limitations

A recent study explored the therapeutic potential of probiotics—*B. lactis, L. brevis, and Limos lactobacillus fermentum*—in a 5× FAD (five familial AD mutations) transgenic mouse model of AD. These probiotics were administered orally, with a phosphatidylserine-enriched diet serving as the control. Behavioral assessments demonstrated notable improvements in spatial learning and recognition memory in the probiotic-treated mice. Additionally, histopathological analysis revealed a reduction in amyloid-β accumulation, tau (a protein that stabilizes microtubules in neurons) phosphorylation, and microglial activation in brain regions commonly affected by AD. These findings suggest that probiotic supplementation may beneficially alter the gut microbiota and provide neuroprotective effects relevant to AD[49]. In another study, the probiotic *B. animalis* subsp. lactis NJ241 was investigated in a murine model of PD induced by the neurotoxin MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine). The research incorporated behavioral tests, molecular assays, gut microbiota profiling, and SCFA analysis. The results indicated that NJ241 improved motor performance, prevented weight loss and GI dysfunction, and protected dopaminergic neurons from MPTP-induced neurotoxicity. Furthermore, probiotic treatment reduced glial cell activation in the substantia nigra, restored microbial diversity, elevated SCFA production, and enhanced GLP-1 signaling in the colon and brain. Blocking the GLP-1 receptor partially diminished these benefits, highlighting its critical role in mediating the neuroprotective effects of NJ241. This study has emphasized the therapeutic potential of gut-derived GLP-1 modulation in PD management through probiotic intervention[50]. Although human and mouse gut microbiota share similar phylum-level profiles dominated by *Bacteroidetes* and *Firmicutes*, they diverge sharply in species composition, metabolic capacity, and ecological behavior. Mice carry taxa rarely found in humans, including Deferribacteres (such as *Mucispirillum schaedleri*) and segmented filamentous bacteria, which have notable immune-modulatory effects. Comparative analyses using 16S rRNA and metagenomic approaches indicate that, despite overlap in general, their relative abundances vary substantially across mouse strains, suppliers, and sequencing methodologies. Deep metagenomics further shows very limited gene-level similarity, despite broad functional overlap. Moreover, only a fraction of murine gut species have been cultured, underscoring persistent gaps in microbiome characterization[51]. The impact of probiotics on mood-related symptoms has also been explored in human models. A study evaluated the effects of *L. helveticus* R0052 and *B. longum* R0175 on the gut microbiota of individuals with mild anxiety using the Simulator of the Human Intestinal Microbial Ecosystem model. Over a two-week intervention period, the probiotics promoted a favorable microbial profile by increasing *Lactobacillus* and *Olsenella* and reducing *Lachnospira* and *Escherichia*-Shigella. Additionally, ammonia levels declined, while levels of acetic acid and total SCFAs increased. Immunological assays revealed elevated levels of anti-inflammatory cytokines (IL-6 and IL-10) and reduced tumor necrosis factor-alpha. These results support the concept of psychobiotics[52]. Psychobiotics are a class of probiotics, prebiotics, or microbial-derived compounds that, when consumed in adequate amounts, can produce mental health benefits by modulating the GBA and influencing mechanisms such as neurotransmitter synthesis, inflammation, stress regulation, and neural signaling. However, most human studies have tested probiotics in healthy individuals, with only a minority involving clinically diagnosed psychiatric patients, limiting conclusions about efficacy in disease contexts. Evidence remains inconsistent due to heterogeneity in strains, doses, study durations, and assessment tools, as well as factors such as timing of administration and dietary differences. While some psychobiotics improve mood or enhance antidepressant response, personalized, multi-strain formulations and standardized trial designs are increasingly necessary[53]. Furthermore, the immunoregulatory effects of probiotics have been investigated in autoimmune disease models. In rat models of experimental autoimmune myasthenia gravis and experimental autoimmune encephalomyelitis (EAE), probiotic administration led to reduced disease severity. In experimental autoimmune myasthenia gravis, neuromuscular junctions were preserved, while in EAE, spinal cord myelin integrity was maintained. Treatment also resulted in increased levels of transforming growth factor-beta (TGF-β) and regulatory T cells in circulation, alongside enhanced immunomodulatory maturation of dendritic cells. These findings suggest that specific probiotic strains may offer therapeutic benefits in autoimmune disorders by promoting anti-inflammatory immune responses[52]. A meta-analysis has indicated that probiotics could help prevent and manage EAE, but evidence is based solely on animal

studies; no eligible human trials were available. Although clinical studies exist, they did not meet inclusion criteria due to their short duration, which limits the evaluation of long-term probiotic effects[54]. The concept of postbiotics has evolved with varying definitions. Initially, Zhong et al. described postbiotics as metabolic byproducts generated by probiotic microorganisms that confer health benefits to the host[55]. In 2019, the International Scientific Association of Probiotics and Prebiotics offered a more precise definition: "a preparation of inanimate microorganisms and/or their components that confers a health benefit on the host"[56]. Evidence from in vitro, animal, and human studies shows that postbiotics can elicit clinically relevant effects. However, their diversity in microbial origin, inactivation methods, delivery matrices, and functional properties complicates classification. A standardized definition is needed to ensure scientific clarity and regulatory consistency[57]. Although studies have suggested potential benefits, especially for GI and immune-related conditions, findings are inconsistent. Moreover, most studies lack microbiota profiling, limiting mechanistic insight. Rigorous, well-designed clinical trials integrating multi-omics approaches are essential to validate postbiotics as safe and effective functional health-promoting agents[58]. Postbiotic research and development focus on three core elements: clearly defining the major classes of postbiotic preparations, linking each class to its physiological mechanisms of action, and selecting appropriate discovery strategies to identify and optimize effective candidates. These strategies are commonly described as top-down (starting from observed clinical or biological effects), bottom-up (starting from specific molecules or mechanisms), or hybrid approaches that combine both paths to accelerate translation and improve reproducibility (Table 1)[57]. Analytical tools such as nuclear magnetic resonance (NMR)[59], gas chromatography, and liquid chromatography are highlighted for metabolite characterization[60]. The table also outlines key advantages such as greater stability[61], targeted bioactivity[62,63], improved safety, and underscores why postbiotics represent a promising next-generation therapeutic modality[64].

2. BIOTECHNOLOGICAL OPPORTUNITIES

2.1. Tools for mapping microbiota-GBA interactions

2.1.1. Metagenomics

Advances in omics technologies have significantly enhanced our understanding of the microbiota-GBA. Among these technologies, metagenomics plays a crucial role by allowing comprehensive analysis of entire microbial communities through techniques such as *16S rRNA* gene sequencing and whole-genome shotgun sequencing. This approach enables researchers to profile the taxonomic diversity, structural complexity, and functional capabilities of gut microbiota. Metagenomic data have been vital in reframing disease models—such as those for IBD and bacterial vaginosis—by framing them as ecological imbalances involving shifts in microbial communities rather than infections caused by single pathogens. Metagenomics also supports the keystone pathogen hypothesis, which suggests that specific low-abundance microbes can disproportionately disrupt community stability and contribute to disease pathogenesis, particularly in oral and GI environments. Additionally, it provides insights into microbial communication systems like quorum sensing, aiding the development of targeted antimicrobial and anti-biofilm strategies[65]. For example, *Clostridioides* difficile infection (CDI) commonly arises from antibiotic-induced dysbiosis, where the loss of protective microbial diversity allows for opportunistic overgrowth of *C. difficile*. High-throughput sequencing techniques have revealed that CDI patients typically exhibit decreased levels of beneficial bacterial families—such as *Bacteroidaceae*, *Lachnospiraceae*, and *Ruminococcaceae*—and elevated levels of opportunistic pathogens like *Enterobacteriaceae*. Thus, metagenomic tools have become essential for understanding CDI pathogenesis and guiding microbiota-based therapeutic interventions[66]. Moreover, metagenomics can detect virulence factors and antibiotic resistance genes that are often missed by conventional diagnostics. This capability is particularly valuable for precision medicine, as it enables the integration of microbiome profiles into drug development pipelines and regulatory frameworks. Accordingly, it may help prevent or mitigate complications arising from dysbiosis[67].

2.1.2. Metabolomics

Metabolomics is a powerful analytical approach that investigates small molecules and metabolites produced during metabolic processes. It provides real-time insights into the functional dynamics of host-microbiota interactions and the broader metabolic landscape, offering valuable biomarkers for both health monitoring and disease diagnosis. Common analytical techniques used in metabolomics include NMR spectroscopy, which provides detailed structural information, and mass spectrometry coupled with chromatography, which enables sensitive and precise metabolite identification. By profiling metabolic signatures, researchers can link specific metabolites to complex conditions such as obesity, type 2 diabetes, and GI disorders, thus facilitating the development of targeted analyses are increasingly applied to the GBA, where

Table 2. A concise overview of postbiotic classification, mechanisms, discovery approaches, and analytical methods, and key advantages

Category	Subcategories/key elements	Examples/nNotes	Ref.
Terminology	Postbiotics, metabiotic, para probiotics, ghost probiotics	Bioactive molecules produced by or released from microorganisms	[49]
	By molecular weight: Low vs. high MW	SCFAs, peptides, exopolysaccharides	
	By structure: peptides, polysaccharides, teichoic acids, lipids	p40, p75, galactose-rich polysaccharides, plasmalogens	
	By composition: Carbohydrates, proteins, lipids, vitamins	SCFAs, B vitamins, muropeptides	
	By function: anti-inflammatory, anti-oxidant, metabolic, immunomodulatory, antimicrobial	Anti-obesogenic and antihypertensive actions	
	Modulation of gut microbiota	Antimicrobial activity, quorum sensing interference	
	Enhancement of epithelial barrier	Improved mucosal integrity and reduced apoptosis	
	Immune modulation	Regulation of Th1/Th2 balance, cytokine production (IL-10, IFN-γ)	
	Metabolic modulation	SCFAs in lipid metabolism, glucose tolerance	
	Systemic signaling	Nervous system interactions, extracellular vesicle-me-diated effects	
	Top-down: phenotype-based screening	Requires culture collections; limited by cultivability	
	Bottom-up: genome/mining and in silico prediction	AI/ML tools; dependent on metabolite databases	
	Hybrid: combines both approaches	Ideal but requires multidisciplinary expertise	
Analytical tools	NMR: structural/kinetic data, low sensitivity	Useful for organic acids, LTA profiling	[51,52]
	GC-MS: volatile/semi-volatile metabolites	Identifies organic acids, fatty acids, antimicrobial Compounds	
	LC-MS: broad metabolite coverage Combined MS platforms	Detects peptides, vitamins, unannotated metabolites Multimodal detection of acids, lipids, vitamins	
Challenges	Broad/ambiguous definition, limited probiotic species, incomplete metabolite databases	Need for integrated multi-omics, AI/ML, and interdisciplinary research	[49]
	Enhanced Stability	Resistant to heat, pH changes, and oxygen, maintaining structural and functional integrity. Do not require cold-chain storage; compatible with capsules, powders, and functional foods; allow predictable dosing and prolonged shelf life	[53]
	Targeted Molecular Action	Postbiotics provide precise immunomodulatory effects via defined biochemical pathways. Butyrate activates GPR109A and inhibits HDACs → induces Tr1 cells → increases IL-10 → reduces inflam-mation; useful for chronic inflammatory conditions	[54,55]
	Improved Safety Profile	Postbiotics avoid risks associated with viable microbes. No replication, infection risk, or horizontal gene transfer; bioactive molecules (SCFAs, peptides, cell-wall components) retain therapeutic activity; suitable for immunocompromised patients	[56]

microbiome-informed therapeutic interventions[68]. Moreover, integrative metabolomics-microbiome pathways involving tryptophan-kynurenine, indole derivatives, and GABA are implicated in depression and neurodegeneration[69].

2.1.3. Multi-omics

Multi-omics approaches, which integrate data from metagenomics, meta-transcriptomics, metabolomics, and glycomics, provide a comprehensive view of microbial functions and interactions within the host. These approaches enable the discovery of diagnostic biomarkers and therapeutic targets, supporting the advancement of precision medicine. Hybrid sequencing technologies, combining short- and long-read platforms, further enhance taxonomic resolution and genome assembly accuracy. Short reads offer high-throughput profiling, while long reads are essential for resolving repetitive or complex genomic regions. The integration of patient metadata, such as clinical history and lifestyle factors, further refines the interpretability and relevance of microbiome studies. Bioinformatics tools such as Quantitative Insights into Microbial Ecology play a critical role in analyzing microbial community structure, function, and metabolomic profiles. Accurate microbial identification relies on high-quality reference databases such as NCBI Nt (nucleotide) and Greengenes, which offer annotated, chimera-checked, full-length 16S rDNA gene sequences in standard alignment formats[70]. Additionally, sequence alignment tools like BLAST and Bowtie facilitate optimal data comparison and annotation[71].

2.1.4. ML in microbiome research

ML methods have become central to deciphering high-dimensional microbiome datasets, each offering complementary strengths for biomarker discovery and disease stratification. Ensemble techniques, such as Random Forests, provide robust nonlinear classification and feature ranking, supporting the identification of discriminatory taxa within complex ecological systems[72]. Linear models with regularization, such as Logistic Regression, LASSO[73] and Elastic Net yield sparse, interpretable signatures that are well-suited for clinical translation. Support vector machines[74] and gradient boosting classifiers (e.g., XGBoost and LightGBM)[75] further enhance predictive performance in large metagenomic datasets, while partial least squares-discriminant analysis enables biologically meaningful integration of microbiome and host-derived multi-omics features[76]. Neural networks and deep-learning architectures capture hierarchical, nonlinear relationships in sequencing and metabolomic data. Additionally, unsupervised methods such as k-means, hierarchical clustering, and autoencoders reveal hidden community structures and patient subgroups[77,78]. Despite these advances, significant analytical challenges remain. Overfitting, dataset-specific biases, and poor cross-cohort reproducibility continue to impede the deployment of microbiome-based diagnostics. The SIAMCAT—a modular R framework—addresses these limitations through standardized preprocessing, confounder testing, normalization, feature selection, and transparent model interpretation. When applied to ulcerative colitis datasets, Statistical Inference of Associations between Microbial Communities and Host Phenotypes identified disease-associated taxa and achieved strong intra-study performance (AUROC ≈ 0.84) using Elastic Net, LASSO, and Random Forest classifiers. However, a meta-analysis of 10,803 samples revealed significant declines in performance when models were applied across different studies, demonstrating that confounders such as geography can inflate accuracy. Enhancing control augmentation substantially improved cross-study portability, emphasizing the need for heterogeneous training cohorts and rigorous external validation[79]. These methodological considerations are underscored by findings from a large multicenter 16S rRNA analysis (n = 2247), which demonstrated the strong diagnostic potential of gut microbiome signatures in IBD. After filtering, 1,846 samples and 1,517 phylotypes were modeled, revealing reduced diversity in IBD. A hierarchical partial least squares discriminant analysis strategy distinguished IBD cases from healthy controls (AUC 0.992) and Crohn's disease from ulcerative colitis (AUC 0.988), achieving an overall accuracy of 0.936. Altogether, these studies illustrate the promise of ML-driven microbiome analytics while highlighting the necessity of standardized pipelines, confounder control, and multi-cohort validation for clinical translation[80].

2.2. Biotechnological innovations

2.2.1. CRISPR-Cas9 and synthetic biology applications

Advances in synthetic biology and CRISPR-Cas9 editing have enabled the rational engineering of probiotic chassis such as EcN to enhance therapeutic functions and metabolic outputs relevant to GBA modulation and inflammatory disorders. CRISPR-Cas9 allows the precise removal of harmful genes and the integration of therapeutic modules, while synthetic genetic circuits enable EcN to act as biosensors that detect intestinal inflammation and conditionally release anti-inflammatory agents such as IL-10. Moreover, the expression of heterologous pathways supports the production of bioactive metabolites including SCFAs, GABA, and GLP-1, which modulate immune signaling

Table 3. ML Methods in Microbiome Research: Properties, Strengths, and challenges

ML method	Type	Key properties	Strengths/ advantages	Limitations/ challenges	Example	Ref.
Random Forest (RF)	Supervised (ensemble-tree)	Many decision trees, bootstrapped voting	Robust to noise; handles nonlinear relationships; reasonable performance with moderate sample sizes; provides feature importance ranking	Risk of overfitting; limited interpretability compared to simple linear models; sensitive to class imbalance	Used successfully for functional prediction from microbiome data	[64]
Logistic Regression/ LASSO / Elastic Net	Supervised (linear, penalized)	Penalizes coefficients to avoid overfitting; selects a sparse set of features	Highly interpretable; feature selection; efficient even with high-dimensional, sparse data	Assumes linear relationships; may fail to capture complex nonlinear interactions common in microbiome data	Reviewed as standard baseline algorithms for microbiome-trait prediction	[66]
Support Vector Machine (SVM)	Supervised (kernel-based)	Maximizes margin between classes; handles high-dimensional data	Effective even with small datasets; handles non-linear class separation (with kernels)	Requires careful parameter tuning; less intuitive interpretability; may struggle with extreme sparsity	Frequently cited in ML-microbiome studies as a classifier option	[66]
Gradient Boosting Machines (e.g., XGBoost / LightGBM)	Supervised (boosted trees)	Sequentially builds trees to correct previous errors; tuning via many hyperparameters	High predictive accuracy; handles complex interactions; can manage mixed feature types	High risk of overfitting; complexity and harder interpretability; tuning-intensive	Emerging use in gut-microbiome classification tasks	[67]
Sparse PLS-DA (sPLS-DA)	Supervised (dimension reduction + feature selection)	Reduces dimensionality while selecting discriminatory features	Especially suited for high-dimensional, sparse microbiome data; balances feature reduction and interpretability	Performance depends on good tuning; may miss nonlinear associations; sensitive to data sparsity and compositionality	Applied in IBD diagnosis from fecal microbiota	[68]
Neural Networks/ Deep Learning	Supervised or unsupervised	Multiple layers learn complex nonlinear relationships automatically	Powerful for large, complex datasets and multi-omics; can detect subtle patterns that broader methods miss	Requires large sample sizes; prone to overfitting; poor interpretability (black box); data-hungry	Growing interest; review highlights both potential and obstacles	[70]
Unsupervised methods (k-means clustering, hierarchical clustering, autoencoders)	Unsupervised	Grouping or dimensionality reduction without labels	Useful to explore hidden structure or subtypes; hypothesis-generating; can reveal novel microbiome-based clusters	Results may not correspond to biological/clinical categories; sensitive to parameter choice and data preprocessing	Used for stratification, community analysis, and exploratory studies	[69]

and neuroendocrine responses. Building on this foundation, one study systematically optimized EcN's native cryptic plasmids pMUT1 and pMUT2 to establish robust, antibiotic-free expression platforms for engineered probiotics[10]. Using CRISPR/Cas9-mediated plasmid curing combined with a functional dissection of coding sequences, the authors removed non-essential elements to generate the minimized plasmids pMUT1NRΔ and pMUT2HBCΔ. These engineered plasmids retained segregation stability for up to 13 days without selection, markedly outperforming the commercial vector pCOLADuet-1, which—under the same selection-free conditions—was lost within just 12 hours. After removing the antibiotic-resistance markers via FLP/FRT recombination (a site-specific DNA recombination system used to precisely modify a genome under controlled in vivo conditions), the authors introduced synthetic σ70/σ38-hybrid promoter libraries that allowed tunable control of expression strength; one such promoter, P1, raised heparinase III production to 5,400 U/L, 210% of control levels. Applying this same antibiotic-free platform to the biosynthesis of ectoine—a heterocyclic, stress-protective amino acid derivative that accumulates in halophilic bacteria and some plants to confer tolerance to high salinity and temperature—likewise yielded superior production compared with conventional systems."[10]. Moreover, CRISPR-Cas systems have been combined with other technologies for developing this method. The CRISPR-Cas9 system, when combined with the Cre/lox system, could enhance the versatility and flexibility of gene editing while minimizing errors. Following chromosomal integration of a vector containing the expression cassettes, Cre recombinase excises the integrated replicon and selectable markers positioned between lox66 and lox71 sites. When the engineered functions are no longer required, the CRISPR-Cas9 system targets and removes the expression cassettes, thereby restoring the native genetic state of the host[81]. Similarly, the type V-K CRISPR-Cas system and transposons improve efficient and specific insertion of large DNA fragments without requiring double-strand breaks (DSBs) and are not dependent on the homologous recombination repair process. For instance, Cas12k recognizes specific sequences, and Tn7-like transposons insert exogenous gene fragments into the genome[82]. Also, the CRISPR-Cas12a (Cpf1) system applies the RecE/T system, which increases homologous recombination activity, facilitating robust expression of Cas12a (Cpf1) and enabling the editing of multiple genes and large DNA fragments. In this combined method, the genome is cleaved by crRNA-directed Cas12a (Cpf1), resulting in DSBs. These DSBs are then repaired using the RecE/T recombination system, which involves both single-stranded DNA and double-stranded DNA recombination[83]. Moreover, the CRISPR-dCas9 system combined with base editors can improve multiplex gene editing efficiency without inducing DSBs, which is independent of the homologous recombination repair process. Multiple single-guide RNAs are used to localize the genome of *Bacillus subtilis*, while cytosine base editors are employed to achieve cytosine-to-thymine base-shift mutations within the target site activity window[84].

2.2.2. Biocontainment through genetic kill switches

To address safety concerns associated with engineered probiotics, synthetic biology has introduced genetic "kill switches", programmable mechanisms that ensure microbes self-destruction after delivering their therapeutic payload. Despite the potential of the "kill switches", maintaining the stability of these kill switches remains a challenge due to evolutionary pressures that may favor escape mutants. Recent advancements have led to the development of two CRISPR-based kill switch systems in EcN: one activated by a chemical input and another activated by a combination of chemical and thermal signals. To improve reliability, researchers have employed multiple stabilization strategies, such as redundant circuit designs, modulation of the bacterial SOS response, maintenance of plasmid-free genes, and the use of competitive bacterial strains to prevent overgrowth. In preclinical mouse models, these systems effectively eliminate engineered strains in vivo, even after excretion, demonstrating their promise for safe deployment in future microbial therapies[85]. However, engineering limitations and biocontainment challenges persist. Rottinghaus et al. have provided important insights into why the translation of engineered probiotics into safe clinical therapies remains challenging. Their study shows that although CRISPR-based kill-switch systems can, in principle, tightly control the survival of engineered bacteria, these systems are far less stable in real biological environments than in controlled laboratory settings. The authors created two kill-switch designs and applied several strategies to strengthen genetic stability, including redundant Cas9/gRNA modules, removal of SOS-related mutagenesis pathways, elimination of antibiotic selection, and introduction of competition within the microbial niche. Despite these innovations, escape mutants have still emerged under gut-like growth conditions, and even the most optimized designs have maintained reliable containment for only a few weeks. The study has also demonstrated that promoter instability and stress-induced DNA repair responses are major drivers of kill-switch failure, while horizontal gene transfer remains a meaningful biosafety concern. Overall, the research highlights that engineered

microbes must be designed with evolution-resistant, multilayered biocontainment systems before they can be safely used as living therapeutics in humans[85].

2.2.3. Encapsulation technologies and symbiotic development

Encapsulation technologies, including micro-encapsulation and nanoencapsulation, are being employed to improve the viability and targeted delivery of probiotics. These techniques protect beneficial microbes from harsh GI conditions, such as stomach acidity and bile salts, enhancing their survival and efficacy upon reaching the intestine. A study assessed whether alginate- and pectin-based microencapsulation could enhance the viability of *L. acidophilus* LA-5 and *B. animalis* BB-12 during freeze-drying, 30-day storage at 4°C, and simulated GI exposure. The results showed that encapsulation efficiency exceeded 92%, with uniform particle sizes across the different formulations. Microencapsulation significantly improved the survival of the probiotics compared to free cells, particularly under freeze-drying and GI stress, with pectin capsules offering the greatest protection. Viable counts remained above 10^6 cfu/g after storage. Scanning electron microscopy imaging confirmed successful capsule formation, showing porous structures that allow for controlled release. Overall, alginate and pectin hydrogels provided effective stabilization of probiotics under multiple stress conditions[86]. Furthermore, the formulation of synbiotics—combinations of probiotics with complementary prebiotics—offers synergistic benefits by supporting microbial colonization and activity. These formulations help restore microbial balance and improve health outcomes across a range of disorders[87]. Additionally, postbiotics—non-viable microbial products with bioactive effects—are emerging as a safer, more stable class of next-generation therapeutics and do not rely on live organisms but still provide significant health benefits[88].

3. CHALLENGES AND LIMITATIONS

3.1. Low survival rates of probiotics

Typical daily intake of probiotics ranges between 10^7 and 10^9 CFU/mg[89]. However, the viability of probiotics is significantly compromised by the harsh conditions of the GI tract, including exposure to gastric acid, bile salts, and digestive enzymes[90]. These factors contribute to survival rates as low as 7% to 30% during the passage of probiotics through the GI system[91]. To address this issue, advanced encapsulation technologies have been developed to enhance probiotic stability. Techniques such as covalent conjugation, non-covalent adsorption, and substrate encapsulation help protect bacterial cells from oxidative stress and enable controlled release at specific GI sites. Recent innovations in encapsulation materials include food-grade biopolymers like polysaccharides and proteins, as well as novel substances such as amino acids, polyphenol complexes, and Eudragit. Eudragit is a copolymer of poly(ethyl acrylate, methyl methacrylate, and chloro trimethyl-

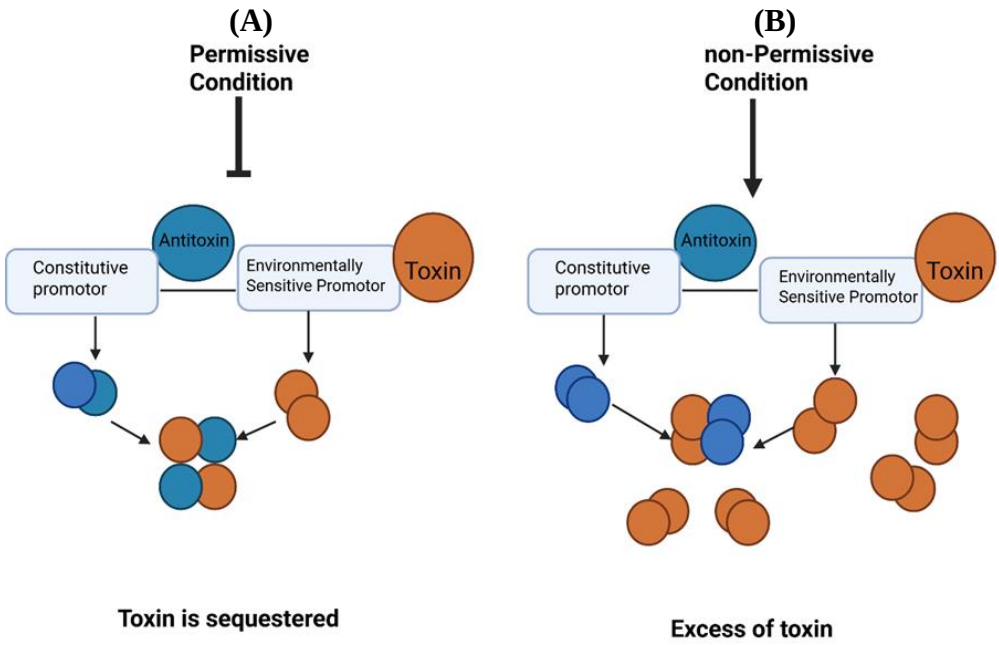

Fig. 3. Kill switch design concept (created with BioRender.com). (A) In a permissive condition, the level of the toxin is low and remains repressed; (B) the effect of environmental change in a non-permissive condition leads to an increase in the level of toxin, which is no longer capable of inhibiting a lethal level of free toxin.

ammonioethyl methacrylate) that is insoluble at physiologic pH. In this context, Eudragit L100-55, a pH-sensitive polymer, dissolves at intestinal pH levels above 5.5 and significantly improves targeted delivery and survival of probiotics[90]. Research has shown that probiotic strains such as *L. acidophilus* CCM 4833, *B. longum* CCM 4990, *B. breve* CCM 3763, *L. reuteri* HFI-LD5, and *L. rhamnosus* HFI-K demonstrate markedly higher survivability when administered as biofilms rather than planktonic forms. Biofilm structures offer enhanced protection in both the acidic environment of the stomach and the alkaline conditions of the proximal small intestine[92,93]. Moreover, *B. subtilis* NCIB3610 has been found to facilitate biofilm formation, thereby providing a protective niche for co-administered probiotics such as *L. plantarum*[94].

3.2. Limited precision in targeted delivery

Current delivery platforms for both probiotics and postbiotics lack specificity in targeting precise regions of the GI tract. For example, postbiotics like SCFAs are often absorbed in the upper GI tract, limiting their intended effects in the colon[95]. To overcome this limitation, nanoparticle-based delivery systems have been developed. One study demonstrated that lysate extracts of *L. paracasei* GMNL-133, when encapsulated in nanoparticles composed of arginine-chitosan and fucoidan, exhibited improved resistance to gastric acid, enhanced mucosal adhesion, and sustained release. These innovations significantly improved the therapeutic potential of postbiotics in gastric cancer models[96].

3.3. Lack of reliable biomarkers

The development and evaluation of psychobiotic interventions are hindered by the absence of validated biomarkers. However, synthetic biology has enabled the engineering of microbial systems that can both detect and respond to disease states. A notable example is i-ROBOT, an engineered EcN strain designed to sense and treat IBD. In a murine colitis model, i-ROBOT responded to thiosulfate levels by activating a heritable base-editing mechanism, producing visible readouts and secreting the immunomodulatory protein AvCystatin, a recombinant cysteine protease inhibitor derived from the filarial nematode Acanthocheilonema viteae. This multifunctional design illustrates the potential for diagnostic and therapeutic integration in microbial therapeutics[97].

4. FUTURE PROSPECTIVE

Future research should be focused firstly on delivery precision and probiotic viability, as these are critical bottlenecks. Many current formulations exhibit low survival rates throughout the GI tract and provide only limited, site-specific exposure. Engineering strategies such as micro- and nanoencapsulation and enteric and pH-responsive coatings such as Eudragit® L100-55, which is an anionic copolymer of methacrylic acid and ethyl acrylate that becomes ionized above pH 5.5 and is commonly used as an enteric coating to protect drugs from degradation in acidic gastric conditions. Other examples are biofilm- or matrix-based delivery systems and nano-enabled protective platforms that can enhance resistance to GI stress, improve mucosal adhesion and retention, and support more controlled and sustained release[98,99]. Second, for engineered probiotics, biocontainment and evolutionary stability are vital for clinical translation. Given that kill-switch performance can deteriorate under gut-like selection pressures, there is a clear need for multilayer, evolution-resistant containment systems (e.g., redundant and orthogonal safety circuits), together with explicit consideration of horizontal gene transfer risks[85]. Third, we found that biomarkers and the integration of multi-omics with ML are important, medium-term building blocks for precision medicine. These tools can help target the appropriate pathways, identify which patients are most likely to respond, and keep track of safety more intelligently. However, microbiome-based ML models often perform well on the original dataset but lose accuracy when tested on independent cohorts. This makes it essential to carefully control for confounders, address batch effects, and rely on strong, external validation rather than single-cohort performance alone[79]. Finally, personalized synbiotics are framed as a longer-term, high-precision goal that depends on three pillars: validated biomarkers, targeted/reliable delivery, and proven biosafety. Using high-throughput sequencing and microbiome profiling, patient-specific microbial imbalances could be translated into tailored synbiotic formulations. These formulations would not only rebalance the gut microbiota but also selectively enhance key functions (e.g., SCFA production, mucosal immunity, and serotonin/GABA-linked neural pathways), shifting the field from generic supplements toward mechanism-based, individualized microbiome therapy[100].

5. CONCLUSION

The gut microbiome is recognized as a key regulator of human physiology through bidirectional communication of the GBA, integrating neural, immune, and metabolic pathways. Disruption in this network is significantly associated with inflammatory, metabolic, and neurodegenerative conditions, motivating the development of microbiome-based interventions. Advances in high-resolution meta-genomics, multi-omics profiling, synthetic biology, and genome engineering, including CRISPR-based tools,

have improved the field from traditional probiotics to next-generation modalities such as NGPs, engineered probiotics, and postbiotics, which are designed to achieve more targeted health outcomes. However, most strong evidence arises from animal studies, whereas human trials are fewer, more heterogeneous, and often yield modest or inconsistent benefits. Differences in microbial ecology, host biology, environmental exposures, and study design contribute to poor reproducibility across cohorts. Notably, accumulating multi-omics evidence indicates that shifting microbial composition alone is rarely sufficient. Functional evidence, such as metabolite flux, pathway activity, and interspecies interactions, may be more predictive of both disease outcome and therapeutic responses than just taxonomic abundance. In this context, postbiotics offer advantages in terms of safety, stability, and standardization. However, they may not reproduce the adaptive and dynamic signaling of live microbial communities, indicating a need for hybrid or staged strategies that combine ecological modulation with targeted effector delivery. For microbiome-based therapies to evolve into reliable GBA interventions, several challenges must be addressed. These challenges include improving the genetic stability of engineered strains, establishing robust biocontainment measures including multilayer kill-switch systems, minimizing off-target immune responses, and managing issues related to horizontal gene transfer and unintentional persistence. Progress will also depend on integrating multi-omics datasets with interpretable AI/ML models that can generalize across cohorts, alongside standardized manufacturing and harmonized regulatory pathways. Future clinical studies should prioritize mechanistic endpoints, transparently report negative and null results, and adopt multicenter longitudinal designs to capture population-level variability and enable patient stratification. Overall, microbial science is shifting from merely observing microbiome associations to developing mechanism-driven, engineered, and personalized therapeutics. With coordinated interdisciplinary efforts across microbiology, neuroscience, synthetic biology, computational modeling, and regulatory science, microbiome-based interventions have the potential to evolve from experimental promise into clinically reproducible strategies for modulating the GBA in chronic systemic and neurological disease.

DECLARATION

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Generative AI and AI-assisted technologies

We applied “Consensus Ai” for finding articles and “SciSpace Ai” for editing and paraphrasing the manuscript.

Ethical approval

All the experimental procedures in this study were conducted in accordance with the principles of the Helsinki Declaration and approved by the Research Ethics Committee of Hamadan University of Medical Sciences, IRAN (ethical code: IR.UMSHA.REC. 1399.524).

Consent to participate

Not applicable.

Consent for publication

Not applicable.

Authors’ contributions

MF: conceptualization, data curation, investigation, methodology, writing–original draft preparation; AM: writing–review & editing; MS and EM: investigation; RD: data curation; MHM: project administration, writing–review & editing; RN: project administration.

Data availability

All relevant data can be found within the manuscript.

Competing interests

The authors declare that they have no competing interests.

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REFERENCES

1. Longo S, Rizza S, Federici M. Microbiota-gut-brain axis: Relationships among the vagus nerve, gut microbiota, obesity, and diabetes. Acta diabetol. 2023;60(8):1007-17.
2. Kargbo RB. Microbiome–gut–brain axis modulation: New approaches in treatment of neuropsychological and gastrointestinal functional disorders. ACS Med Chem Lett. 2023;14(6):692-5.
3. Ashique S, Mohanto S, Ahmed MG, Mishra N, Garg A,

Chellappan DK, et al. Gut-brain axis: A cutting-edge approach to target neurological disorders and potential synbiotic application. Heliyon. 2024;10(13):e34092.
4. Thursby E, Juge N. Introduction to the human gut microbiota. Biochem J. 2017;474(11):1823-36.
5. Kustrimovic N, Balkhi S, Bilato G, Mortara L. Gut microbiota and immune system dynamics in Parkinson's and Alzheimer's diseases. Int J Mol Sci. 2024;25(22):12164.
6. Hadrich I, Turki M, Chaari I, Abdelmoula B, Gargouri R, Khemakhem N, et al. Gut mycobiome and neuropsychiatric disorders: Insights and therapeutic potential. Front Cell Neurosci. 2025;18:1495224.
7. Zhou P, Chen C, Patil S, Dong S. Unveiling the therapeutic symphony of probiotics, prebiotics, and postbiotics in gut-immune harmony. Front Nutr. 2024;11:1355542.
8. Yang J, Qin S, Zhang H. Precise strategies for selecting probiotic bacteria in treatment of intestinal bacterial dysfunctional diseases. Front Immunol. 2022;13:1034727.
9. Westfall S, Carracci F, Estill M, Zhao D, Wu Q-l, Shen L, et al. Optimization of probiotic therapeutics using machine learning in an artificial human gastrointestinal tract. Sci Rep. 2021;11(1):1067.
10. Zhou S, Zhao L, Zuo W, Zheng Y, Zhang P, Sun Y, et al. Minimizing endogenous cryptic plasmids to construct antibiotic-free expression systems for Escherichia coli Nissle 1917. Synth Syst Biotechnol. 2024;9(1):165-75.
11. Foo GW, Uruthirapathy AS, Zhang CQ, Batko I, Heinrichs DE, Edgell DR. Genetic entanglement enables ultrastable biocontainment in the mammalian gut. ACS Synth Biol. 2025;14(9):3696-708.
12. Sender R, Fuchs S, Milo R. Revised estimates for the number of human and bacteria cells in the body. Plos Biol. 2016;14(8):e1002533.
13. Kim S, Kim H, Yim YS, Ha S, Atarashi K, Tan TG, et al. Maternal gut bacteria promote neurodevelopmental abnormalities in mouse offspring. Nature. 2017;549(7673):528-32.
14. Rutsch A, Kantsjö JB, Ronchi F. The gut-brain axis: How microbiota and host inflammasome influence brain physiology and pathology. Front Immunol. 2020;11:604179.
15. Silva YP, Bernardi A, Frozza RL. The role of short-chain fatty acids from gut microbiota in gut-brain communication. Front Endocrinol. 2020;11:25.
16. Cherbut C, Ferrier L, Rozé C, Anini Y, Blottière H, Lecannu G, et al. Short-chain fatty acids modify colonic motility through nerves and polypeptide YY release in the rat. Am J Physiol. 1998;275(6):G1415-22.
17. Vinolo MA, Rodrigues HG, Nachbar RT, Curi R. Regulation of inflammation by short chain fatty acids. Nutrients. 2011;3(10):858-76.
18. He J, Zhang P, Shen L, Niu L, Tan Y, Chen L, et al. Short-chain fatty acids and their association with signalling pathways in inflammation, glucose and lipid metabolism. Int J Mol Sci. 2020;21(17):6356.
19. May KS, den Hartigh LJ. Modulation of adipocyte metabolism by microbial short-chain fatty acids. Nutrients. 2021;13(10):3666.
20. Cao S, Liu M, Han Y, Li S, Zhu X, Li D, et al. Effects of saponins on lipid metabolism: The gut–liver axis plays a key role. Nutrients. 2024;16(10):1514.
21. Dicks LM. Gut bacteria and neurotransmitters. Microorganisms. 2022;10(9):1838.
22. Chen Y, Xu J, Chen Y. Regulation of neurotransmitters by the gut microbiota and effects on cognition in neurological disorders. Nutrients. 2021;13(6):2099.
23. Parthasarathy A, Cross PJ, Dobson RC, Adams LE, Savka MA, Hudson AO. A three-ring circus: Metabolism of the three proteogenic aromatic amino acids and their role in the health of plants and animals. Front Mol Biosci. 2018;5:29.
24. Swer NM, Venkidesh BS, Murali TS, Mumbrekar KD. Gut microbiota-derived metabolites and their importance in neurological disorders. Mol Biol Rep. 2023;50(2):1663-75.
25. Lee JH, Lee J. Indole as an intercellular signal in microbial communities. FEMS Microbiol Rev. 2010;34(4):426-44.
26. Wang Z, Klipfell E, Bennett BJ, Koeth R, Levison BS, Dugar B, et al. Gut flora metabolism of phosphatidylcholine promotes cardiovascular disease. Nature. 2011;472(7341):57-63.
27. Brunt VE, LaRocca TJ, Bazzoni AE, Sapinsley ZJ, Miyamoto-Ditmon J, Gioscia-Ryan RA, et al. The gut microbiome–derived metabolite trimethylamine N-oxide modulates neuroinflammation and cognitive function with aging. Geroscience. 2021;43(1):377-94.
28. Ji J, Jin W, Liu SJ, Jiao Z, Li X. Probiotics, prebiotics, and postbiotics in health and disease. MedComm. 2023;4(6):e420.
29. Spacova I, Binda S, Ter Haar JA, Henoud S, Legrain-Raspaud S, Dekker J, et al. Comparing technology and regulatory landscape of probiotics as food, dietary supplements and live biotherapeutics. Front Microbiol. 2023;14:1272754.
30. Doron S, Snydman DR. Risk and safety of probiotics. Clin Infect Dis. 2015;60(suppl 2):S129-34.
31. Abouelela ME, Helmy YA. Next-generation probiotics as novel therapeutics for improving human health: Current trends and future perspectives. Microorganisms. 2024;12(3):430.
32. O'Toole PW, Marchesi JR, Hill C. Next-generation probiotics: The spectrum from probiotics to live biotherapeutics. Nat Microbiol. 2017;2:17057.
33. De Filippis F, Esposito A, Ercolini D. Outlook on next-generation probiotics from the human gut. Cell Mol Life Sci. 2022;79(2):76.
34. Jadhav NK, Magdum AB, Shinde KV, Nimbalkar MS. Next-generation probiotics: From traditional strains to personalized therapeutics. Mol Nutr Food Res. 2026:70(1):e70339.
35. Ma J, Lyu Y, Liu X, Jia X, Cui F, Wu X, et al. Engineered probiotics. Microb Cell Fact. 2022;21(1):72.
36. Bischoff SC, Barbara G, Buurman W, Ockhuizen T, Schulzke J-D, Serino M, et al. Intestinal permeability–a new target for disease prevention and therapy. BMC Gastroenterol. 2014;14:189.

37. Alvarez CS, Badia J, Bosch M, Giménez R, Baldomà L. Outer membrane vesicles and soluble factors released by probiotic Escherichia coli Nissle 1917 and commensal ECOR63 enhance barrier function by regulating expression of tight junction proteins in intestinal epithelial cells. Front Microbiol. 2016;7:1981.
38. Fusco A, Savio V, Cimini D, D'Ambrosio S, Chiaromonte A, Schiraldi C, et al. In vitro evaluation of the most active probiotic strains able to improve the intestinal barrier functions and to prevent inflammatory diseases of the gastrointestinal system. Biomedicines. 2023;11(3):865.
39. Chandrasekaran P, Weiskirchen S, Weiskirchen R. Effects of probiotics on gut microbiota: An overview. Int J Mol Sci. 2024;25(11):6022.
40. Michels M, Jesus GFA, Abatti MR, Córneo E, Cucker L, Borges H, et al. Effects of different probiotic strains B. lactis, L. rhamnosus and L. reuteri on brain-intestinal axis immunomodulation in an endotoxin-induced inflammation. Mol Neurobiol. 2022;59(8):5168-78.
41. Yang J, Liang J, Hu N, He N, Liu B, Liu G, et al. The gut microbiota modulates neuroinflammation in Alzheimer's disease: Elucidating crucial factors and mechanistic underpinnings. CNS Neurosci Ther. 2024;30(10):e70091.
42. Barcutean L, Maier S, Burai-Patrascu M, Farczadi L, Balasa R. The immunomodulatory potential of short-chain fatty acids in multiple sclerosis. Int J Mol Sci. 2024;25(6):3198.
43. Garvey M. The association between dysbiosis and neurological conditions often manifesting with chronic pain. Biomedicines. 2023;11(3):748.
44. Góralczyk-Bińkowska A, Szmajda-Krygier D, Kozłowska E. The microbiota–gut–brain axis in psychiatric disorders. Int J Mol Sci. 2022;23(19):11245.
45. Santana PT, Rosas SLB, Ribeiro BE, Marinho Y, De Souza HS. Dysbiosis in inflammatory bowel disease: Pathogenic role and potential therapeutic targets. Int J Mol Sci. 2022;23(7):3464.
46. Erdrich S, Hawrelak JA, Myers SP, Harnett JE. A systematic review of the association between fibromyalgia and functional gastrointestinal disorders. Ther Adv Gastroenterol. 2020;13:1756284820977402.
47. Sanada K, Díez MA, Valero MS, Pérez-Yus MC, Demarzo MM, García-Toro M, et al. Effects of non-pharmacological interventions on inflammatory biomarker expression in patients with fibromyalgia: A systematic review. Arthritis Res Ther. 2015;17:272.
48. Garofalo C, Cristiani CM, Ilari S, Passacatini LC, Malafoglia V, Viglietto G, et al. Fibromyalgia and irritable bowel syndrome interaction: A possible role for gut microbiota and gut-brain axis. Biomedicines. 2023;11(6):1701.
49. Kim YJ, Mun BR, Choi KY, Choi WS. Oral administration of probiotic bacteria alleviates tau phosphorylation, aβ accumulation, microglia activation, and memory loss in 5xFAD mice. Brain Sci. 2024;14(3):208.
50. Dong Y, Qi Y, Chen J, Han S, Su W, Ma X, et al. Neuroprotective effects of bifidobacterium animalis subsp. lactis NJ241 in a mouse model of Parkinson's disease: Implications for gut microbiota and PGC-1α. Mol Neurobiol. 2024;61(10):7534-48.
51. Hugenholtz F, De Vos WM. Mouse models for human intestinal microbiota research: A critical evaluation. Cell Mol Life Sci. 2018;75(1):149-60.
52. De Oliveira FL, Salgaço MK, De Oliveira MT, Mesa V, Sartoratto A, Peregrino AM, et al. Exploring the potential of Lactobacillus helveticus R0052 and Bifidobacterium longum R0175 as promising psychobiotics using SHIME. Nutrients. 2023;15(6):1521.
53. Binda S, Tremblay A, Iqbal UH, Kassem O, Le Barz M, Thomas V, et al. Psychobiotics and the microbiota–gut–brain axis: Where do we go from here? Microorganisms. 2024;12(4):634.
54. Valizadeh S, Majdi Seghinsara A, Maleki Chollou K, Bahadori A, Abbaszadeh S, Taghdir M, et al. The efficacy of probiotics in experimental autoimmune encephalomyelitis (an animal model for MS): A systematic review and meta-analysis. Lett Appl Microbiol. 2021;73(4):408-17.
55. Zhong Y, Wang S, Di H, Deng Z, Liu J, Wang H. Gut health benefit and application of postbiotics in animal production. J Anim Sci Biotechnol. 2022;13(1):38.
56. Salminen S, Collado MC, Endo A, Hill C, Lebeer S, Quigley EM, et al. The international scientific association of probiotics and prebiotics (ISAPP) consensus statement on the definition and scope of postbiotics. Nat Rev Gastroenterol Hepatol. 2021;18(9):649-67.
57. Kumar A, Green KM, Rawat M. A comprehensive overview of postbiotics with a special focus on discovery techniques and clinical applications. Foods. 2024;13(18):2937.
58. Wegh CA, Geerlings SY, Knol J, Roeselers G, Belzer C. Postbiotics and their potential applications in early life nutrition and beyond. Int J Mol Sci. 2019;20(19):4673.
59. Emwas AH, Roy R, McKay RT, Tenori L, Saccenti E, Gowda GA, et al. NMR spectroscopy for metabolomics research. Metabolites. 2019;9(7):123.
60. Perez ER, Knapp JA, Horn CK, Stillman SL, Evans JE, Arfsten DP. Comparison of LC–MS-MS and GC–MS analysis of benzodiazepine compounds included in the drug demand reduction urinalysis program. J Anal Toxicol. 2016;40(3):201-7.
61. Wei L, Wang B, Bai J, Zhang Y, Liu C, Suo H, et al. Postbiotics are a candidate for new functional foods. Food Chem X. 2024;23:101650.
62. Kaisar MM, Pelgrom LR, Van der Ham AJ, Yazdanbakhsh M, Everts B. Butyrate conditions human dendritic cells to prime type 1 regulatory T cells via both histone deacetylase inhibition and G protein-coupled receptor 109A signaling. Front Immunol. 2017;8:1429.
63. Siddiqui MT, Han Y, Shapiro D, West G, Fiocchi C, Cresci GA. The postbiotic butyrate mitigates gut mucosal disruption caused by acute ethanol exposure. Int J Mol Sci. 2024;25(3):1665.
64. Amobonye A, Pillay B, Hlope F, Asong ST, Pillai S. Postbiotics: An insightful review of the latest category in functional biotics. World J Microbiol Biotechnol. 2025;41(8):293.

65. Chaudhari HG, Prajapati S, Wardah ZH, Raol G, Prajapati V, Patel R, et al. Decoding the microbial universe with metagenomics: A brief insight. Front Genet. 2023;14:1119740.
66. Wang L, Chen X, Pollock NR, Villafuerte Gálvez JA, Alonso CD, Wang D, et al. Metagenomic analysis reveals distinct patterns of gut microbiota features with diversified functions in C. difficile infection (CDI), asymptomatic carriage and non-CDI diarrhea. Gut Microbes. 2025;17(1):2505269.
67. Mulcahy-O'Grady H, Workentine ML. The challenge and potential of metagenomics in the clinic. Front Immunol. 2016;7:29.
68. Vernocchi P, Del Chierico F, Putignani L. Gut microbiota profiling: Metabolomics based approach to unravel compounds affecting human health. Front Microbiol. 2016;7:1144.
69. Abildinova GZ, Benberin VV, Vochshenkova TA, Afshar A, Mussin NM, Kaliyev AA, et al. The gut-brain-metabolic axis: Exploring the role of microbiota in insulin resistance and cognitive function. Front Microbiol. 2024;15:1463958.
70. DeSantis TZ, Hugenholtz P, Larsen N, Rojas M, Brodie EL, Keller K, et al. Greengenes, a chimera-checked 16S rRNA gene database and workbench compatible with ARB. Appl Environ Microbiol. 2006;72(7):5069-72.
71. Wu J, Singleton SS, Bhuiyan U, Krammer L, Mazumder R. Multi-omics approaches to studying gastrointestinal microbiome in the context of precision medicine and machine learning. Front Mol Biosci. 2024;10:1337373.
72. Thompson J, Johansen R, Dunbar J, Munsky B. Machine learning to predict microbial community functions: An analysis of dissolved organic carbon from litter decomposition. Plos One. 2019;14(7):e0215502.
73. Zhou YH, Gallins P. A review and tutorial of machine learning methods for microbiome host trait prediction. Front Genet. 2019;10:579.
74. Shalaby M, Farouk M, Khater HA. Data reduction for SVM training using density-based border identification. Plos One. 2024;19(4):e0300641.
75. Yang L, Zhou W, Zhang C, Tang F. A comparative machine learning study identifies light gradient boosting machine (LightGBM) as the optimal model for unveiling the environmental drivers of yellowfin tuna (Thunnus albacares) distribution using SHapley additive explanations (SHAP) analysis. Biology. 2025;14(11):1567.
76. Lê Cao K-A, Boitard S, Besse P. Sparse PLS discriminant analysis: Biologically relevant feature selection and graphical displays for multiclass problems. BMC Bioinformatics. 2011;12:253.
77. Elguoshy A, Zedan H, Saito S. Machine learning-driven insights in cancer metabolomics: From subtyping to biomarker discovery and prognostic modeling. Metabolites. 2025;15(8):514.
78. Zhang J, Che Y, Liu R, Wang Z, Liu W. Deep learning–driven multi-omics analysis: Enhancing cancer diagnostics and therapeutics. Brief Bioinform. 2025;26(4):bbaf440.
79. Wirbel J, Zych K, Essex M, Karcher N, Kartal E, Salazar G, et al. Microbiome meta-analysis and cross-disease comparison enabled by the SIAMCAT machine learning toolbox. Genome Biol. 2021;22(1):93.
80. Kim H, Na JE, Kim S, Kim TO, Park SK, Lee CW, et al. A machine learning-based diagnostic model for Crohn's disease and ulcerative colitis utilizing fecal microbiome analysis. Microorganisms. 2023;12(1):36.
81. Cai MZ, Chen PT. Novel combined Cre-Cas system for improved chromosome editing in Bacillus subtilis. J Biosci Bioeng. 2021;132(2):113-9.
82. Strecker J, Ladha A, Gardner Z, Schmid-Burgk JL, Makarova KS, Koonin EV, et al. RNA-guided DNA insertion with CRISPR-associated transposases. Science. 2019;365(6448):48-53.
83. Zhao N, Li L, Luo G, Xie S, Lin Y, Han S, et al. Multiplex gene editing and large DNA fragment deletion by the CRISPR/Cpf1-RecE/T system in Corynebacterium glutamicum. J Ind Microbiol Biotechnol. 2020;47(8):599-608.
84. Yu S, Price M, Wang Y, Liu Y, Guo Y, Ni X, et al. CRISPR-dCas9 mediated cytosine deaminase base editing in Bacillus subtilis. ACS Synth Biol. 2020;9(7):1781-9.
85. Rottinghaus AG, Ferreiro A, Fishbein SR, Dantas G, Moon TS. Genetically stable CRISPR-based kill switches for engineered microbes. Nat Commun. 2022;13(1):672.
86. Motalebi Moghanjougi Z, Rezazadeh Bari M, Alizadeh Khaledabad M, Amiri S, Almasi H. Microencapsulation of Lactobacillus acidophilus LA-5 and Bifidobacterium animalis BB-12 in pectin and sodium alginate: A comparative study on viability, stability, and structure. Food Sci Nutr. 2021;9(9):5103-11.
87. Markowiak P, Śliżewska K. Effects of probiotics, prebiotics, and synbiotics on human health. Nutrients. 2017;9(9):1021.
88. Aggarwal S, Sabharwal V, Kaushik P, Joshi A, Aayushi A, Suri M. Postbiotics: From emerging concept to application. Front Sustain Food Syst. 2022;6:887642.
89. Ranjha MMAN, Shafique B, Batool M, Kowalczewski PŁ, Shehzad Q, Usman M, et al. Nutritional and health potential of probiotics: A review. Appl Sci. 2021;11(23):11204.
90. Garcia-Brand AJ, Quezada V, Gonzalez-Melo C, Bolaños-Barbosa AD, Cruz JC, Reyes LH. Novel developments on stimuli-responsive probiotic encapsulates: From smart hydrogels to nanostructured platforms. Fermentation. 2022;8(3):117.
91. Yoha KS, Nida S, Dutta S, Moses JA, Anandharamakrishnan C. Targeted delivery of probiotics: Perspectives on research and commercialization. Probiotics Antimicrob Proteins. 2022;14(1):15-48
92. Grossova M, Rysavka P, Marova I. Probiotic biofilm on carrier surface: A novel promising application for food industry. Acta Alimentaria. 2017;46(4):439-48.
93. Klopper KB, Bester E, Deane SM, Wolfaardt GM, Dicks LM. Survival of planktonic and sessile cells of lactobacillus rhamnosus and lactobacillus reuteri upon exposure to simulated fasting-state gastrointestinal conditions. Probiotics Antimicrob Proteins.

2019;11(2):594-603.
94. Yahav S, Berkovich Z, Ostrov I, Reifen R, Shemesh M. Encapsulation of beneficial probiotic bacteria in extracellular matrix from biofilm-forming Bacillus subtilis. Artif Cells Nanomed Biotechnol. 2018;46(sup2):974-82.
95. McClements DJ. Designing biopolymer microgels to encapsulate, protect and deliver bioactive components: Physicochemical aspects. Adv Colloid Interface Sci. 2017;240:31-59.
96. Huang HL, Lai CH, Tsai WH, Chen KW, Peng SL, Lin JH, et al. Nanoparticle-enhanced postbiotics: Revolutionizing cancer therapy through effective delivery. Life Sciences. 2023;337(11):122379.
97. Zou ZP, Du Y, Fang TT, Zhou Y, Ye BC. Biomarker-responsive engineered probiotic diagnoses, records, and ameliorates inflammatory bowel disease in mice. Cell Host Microbe. 2023;31(2):199-212.e5.
98. Kailasapathy K. Microencapsulation of probiotic bacteria: Technology and potential applications. Curr Issues Intest Microbiol. 2002;3(2):39-48.
99. Pan J, Gong G, Wang Q, Shang J, He Y, Catania C, et al. A single-cell nanocoating of probiotics for enhanced amelioration of antibiotic-associated diarrhea. Nat Commun. 2022;13(1):2117.
100. Swanson KS, Gibson GR, Hutkins R, Reimer RA, Reid G, Verbeke K, et al. The International Scientific Association for Probiotics and Prebiotics (ISAPP) consensus statement on the definition and scope of synbiotics. Nat Rev Gastroenterol Hepatol. 2020;17(11):687-701.