

Genome editing and precision engineering of probiotic strains: A narrative review

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ABSTRACT

Probiotics contribute to gut homeostasis, immune regulation, metabolic balance, and host–microbiome signaling, but the clinical performance of conventional strains remains inconsistent because of limited gastrointestinal survival, strain-specific functionality, host-dependent variability, and incompletely understood mechanisms of action. This narrative review examines how genome editing and synthetic biology are being applied to address these constraints in probiotic engineering. We compare six genome-engineering technologies used in probiotic strain development—CRISPR-Cas systems, genome shuffling, zinc-finger nucleases (ZFNs), transcription activator-like effector nucleases (TALENs), base editing, and prime editing—against six criteria: editing precision, capacity for multitrait optimization, scalability, regulatory readiness, clinical maturity and best-suited application. The comparison reflects a literature-based, expert-synthesized assessment rather than a validated quantitative or clinically derived scoring system, and technology maturity is found to vary widely: CRISPR-Cas systems are the most extensively characterized in probiotic hosts, genome shuffling remains a recombination-based strain-improvement approach rather than precision editing, and base and prime editing are still largely at an early, species-dependent stage of development in bacteria. The review also considers translational barriers, including biosafety concerns such as off-target editing and horizontal gene transfer, heterogeneous regulatory classification of engineered strains across jurisdictions, manufacturing and strain-stability constraints, and the current scarcity of controlled human clinical data for genome-edited probiotics as distinct from conventional probiotic supplementation. We conclude that no single technology is sufficient on its own, and that combining complementary editing approaches with multi-omics and computational tools, alongside standardized safety testing and clearer regulatory pathways, will be necessary before precision-engineered probiotics can move from preclinical and early-stage research into validated live biotherapeutics.

1. INTRODUCTION

Probiotics have long been associated with benefits for gut homeostasis, immune regulation, and metabolic health [1]. Adequate delivery of live probiotic organisms can improve nutrient absorption, immune defense, and disease resistance [2,3], and the gastrointestinal microbial ecosystem plays a central role in digestion, immunity, and metabolism [4]; disruption of this ecosystem by diet, antibiotic use, and chronic stress is associated with dysbiosis and with gastrointestinal and systemic illness [5,6]. It is important to note that most of this evidence is strain- and condition-specific rather than a general property of "probiotics" as a category: benefits demonstrated for one *Lactobacillus* or *Bifidobacterium* strain do not necessarily generalize to others,

and the strength of clinical evidence varies considerably across the conditions discussed below.

Certain well-characterized strains, such as *Lactobacillus rhamnosus* and *Bifidobacterium bifidum*, have been reported to support epithelial tight-junction integrity and reduce intestinal permeability, with clinical benefit described in irritable bowel syndrome (IBS) and inflammatory bowel disease (IBD) [7,8]. Because roughly 70% of the body's immune cells reside in gut-associated lymphoid tissue, probiotics can also influence immune tolerance and pathogen recognition through effects on immunoglobulin A (IgA), natural killer (NK) cell activity, and pro- and anti-inflammatory cytokine balance [9–12]. Preliminary evidence further associates specific psychobiotic strains, including *Lactobacillus plantarum* and *Bifidobacterium longum*, with gut–brain axis signaling and neurotransmitter regulation [13–16], and other strains with improved lipid and glucose metabolism [17,18]. Much of this evidence, however, is drawn from small or heterogeneous trials with mixed replication across populations, and should not be read as established for probiotics as a whole.

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Despite this evidence base, conventional probiotics show inconsistent clinical performance. Many commercial strains tolerate gastric acid and bile poorly, which limits intestinal viability and colonization [19,20], and efficacy is strongly strain- and host-microbiome-dependent [21]. Formulation strategies such as microencapsulation and nano-coating improve delivery but do not resolve the underlying biological constraints on stress tolerance, adhesion, or metabolic output. Regulatory classification compounds these limitations: most probiotics are marketed as dietary supplements rather than as drugs or live biotherapeutic products, which permits variability in product quality, dosing, and substantiated claims [22], and rare but serious adverse events, including bacteremia and sepsis in immunocompromised patients, have been reported [23].

These constraints originate at the genetic level—the traits that determine acid tolerance, adhesion, and metabolite output are fixed properties of a strain's genome—and are therefore not fully addressed by strain screening or formulation alone. Genome editing and synthetic biology offer a route to intervene directly at this level, enabling rational, targeted modification of the genetic determinants of survival, colonization, immune interaction, and metabolite production, rather than relying on the trial-and-error screening that constrains conventional strain development [24,25]. For clarity, this review distinguishes several terms that are often used loosely in the literature. An “engineered probiotic” is a strain deliberately modified using recombinant-deoxyribonucleic acid (DNA) or genome-editing tools to acquire or enhance a defined trait. A “live biotherapeutic product” (LBP) is the regulatory category used by agencies such as the U.S. Food and Drug Administration (FDA) for biological products containing live organisms intended to treat, cure, or prevent disease, and is distinct from a probiotic marketed as a food or dietary supplement. “Next-generation” or “synthetic” probiotic refers more broadly to any rationally designed or non-conventional strain, whether or not it carries foreign DNA. “Precision probiotic engineering”, as used in this review, refers specifically to site-directed genome-editing tools [CRISPR-Cas systems, zinc-finger nucleases (ZFNs), transcription activator-like effector nucleases (TALENs), and base and prime editing], as distinct from recombination-based approaches such as genome shuffling or classical mutagenesis. CRISPR-Cas systems, genome shuffling, and base and prime editing each enable different degrees of precision relative to traditional mutagenesis [26,27], and the integration of omics technologies, high-throughput screening (HTS), and artificial intelligence (AI) is increasingly used to accelerate strain optimization and the development of strain-specific, microbiome-informed therapies [28].

1.1. Scope, Objectives, and Structure of this Review

This review critically examines genome editing and synthetic biology as tools for probiotic engineering, with three objectives: (i) to characterize the genetic-level constraints of conventional probiotic strain development that motivate a shift toward precision engineering; (ii) to compare the major genome-engineering technologies—CRISPR-based systems, genome shuffling, ZFNs, TALENs, and base and prime editing—with respect to precision, multitrait optimization capacity, scalability, regulatory readiness, and clinical maturity; and (iii) to assess the translational landscape, including current limitations, technologies with restricted applicability, and a realistic outlook for clinical and regulatory progress. The review is organized to first place probiotic engineering in its biological and historical context, then critically compare current genome-editing technologies, discuss their application to health and disease with clinical and preclinical evidence distinguished where possible, and finally examine regulatory,

manufacturing, and ethical considerations shaping translation. By combining recent technical developments with an explicit account of unresolved issues, this review aims to offer a balanced, evidence-proportionate perspective on the role precision probiotic engineering may play in the future of microbiome-based therapeutics.

2. REVIEW METHODOLOGY

This is a narrative review rather than a systematic or scoping review; no formal registered protocol, meta-analysis, or quantitative synthesis was undertaken. Relevant literature was identified through targeted searches of PubMed, Scopus, and Web of Science (2010–2025, with emphasis on 2018–2025) using combinations of terms including “probiotic,” “engineered probiotic,” “live biotherapeutic,” “CRISPR,” “genome shuffling,” “ZFN,” “TALEN,” “base editing,” “prime editing,” “biosafety,” and “regulatory,” supplemented by hand-searching reference lists of key reviews and, where relevant, regulatory guidance documents from the FDA, European Medicines Agency, and other agencies.

Peer-reviewed primary research and review articles in English were prioritized; conference abstracts, non-peer-reviewed preprints, and sources without accessible methodological detail were generally excluded unless no peer-reviewed alternative existed. Because the literature on probiotic genome editing spans *in vitro*, *in vivo* (animal), and, occasionally, human studies, each finding cited in this review is described according to that distinction (*in vitro*/preclinical vs. animal vs. human/clinical) rather than presented uniformly; no formal quality-appraisal instrument (e.g., GRADE or Cochrane risk-of-bias tools) was applied, and this should be read as a limitation of the synthesis. Comparative judgments of technology “maturity” or “clinical readiness” in this review reflect the reviewers' qualitative synthesis of the literature, using the following working definitions: preclinical/conceptual (demonstrated only *in vitro* or computationally), preclinical *in vivo* (demonstrated in animal models), and early clinical (human safety or efficacy data reported, even if limited); no technology addressed in this review currently has data supporting a “clinically validated” designation in engineered form.

This narrative approach is subject to the usual limitations of non-systematic reviews, including potential selection and publication bias, uneven depth of coverage across probiotic species and editing technologies, and the risk that conclusions will be superseded quickly given the pace of development in this field. Evidence on food-grade probiotics intended for general wellness is discussed separately from evidence on strains developed as candidate live biotherapeutic products, because the two are subject to materially different safety, manufacturing, and regulatory expectations.

2.1. Emergence of Genome Editing and Genome Shuffling in Probiotic Engineering

The scientific field of probiotics experienced a revolution due to genome editing combined with synthetic biology applications. Scientists use modern genetic tools to repurpose probiotics, thus making them more resilient so they can reach higher therapeutic value while becoming suitable for many human health treatments (Fig. 1). CRISPR-Cas technology coupled with synthetic biology approaches provides an unmatched precision capability for biotechnological engineering of therapeutic and commercial applications [24]. The transformation of probiotics into genetically manufactured tools has expanded their potential capabilities to deliver drugs while modifying immune responses and regulating bodily metabolism.

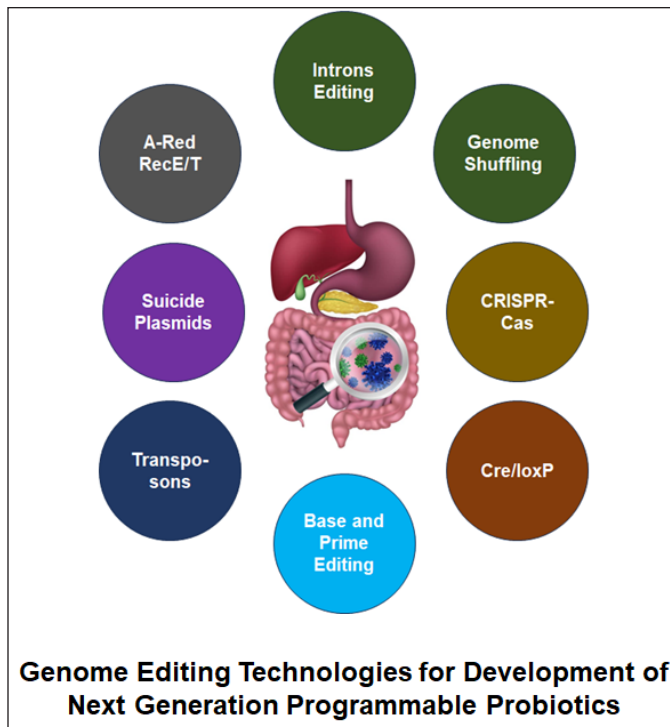


Figure 1. Introduction to genome-engineering tools for developing probiotic strains. The figure provides an overview of six major strategies, including CRISPR-Cas, genome shuffling, ZFNs, TALENs, base editing, and prime editing, explaining their working principles and potential applications to enhance probiotic survival and adhesion, metabolic functions, biosensing, and therapeutic properties. Source: Author-created.

Probiotics have complex health functions that interact through an organized effect on gastrointestinal integrity, immune homeostasis, metabolic homeostasis, and host–microbiome signaling. Probiotic supplementation of the intestines preserves the balance of gut microorganisms and increases digestive efficiency, enhances intestinal nutrient absorption, and prevents the growth of pathogenic microorganisms [4,6,29]. It has been clinically proven that the presence of probiotics improves symptoms and signs of the IBS, IBD, and other gastrointestinal conditions by enhancing epithelial intestinal integrity and functionality and suppressing intestinal inflammation [7]. Strains such as *L. rhamnosus* and *B. bifidum* enhance tight junction integrity, reliably containing intestinal permeability and preventing metabolic and systemic downstream inflammatory and metabolic complications [8].

In addition to gut health, probiotics are highly involved in the immune modulation process, due to the strong connection between the gut microbiota and the gut-associated lymphoid tissue, which harbors most of the immune cells in the body [9]. Immune regulation via probiotics involves an increase in the secretory IgA, the NK activation, and antigen presentation by dendritic cells [10]. Notably, individual types of probiotics inhibit hyperinflammatory reactions, suppressing pro-inflammatory cytokines, including tumor necrosis factor- α and interleukin-6 (IL-6), and promoting anti-inflammatory cytokines, including interleukin-10 (IL-10), to inhibit excessive inflammatory reactions [11,12]. The concerted efforts lead to better results in allergy, autoimmune, and higher-order inflammatory diseases.

The discussion of the use of probiotics in the regulation of neurocognitive and metabolic health of the gut-brain and gut-liver

axis is also becoming more and more evidenced [13,14]. *Lactobacillus plantarum* and *B. longum*, which are psychobiotic strains, regulate neurotransmitter production, including serotonin and γ -aminobutyric acid, which affect mood, stress response, and cognitive performance [15]. At the same time, neuro defense mechanisms against neurodegenerative diseases, including Alzheimer's and Parkinson, involve the reduction of oxidative stress and systemic inflammation by probiotics [16].

The effects on host metabolic regulation are also strong when probiotics are used. The changes in the composition of the gut microbiota have an impact on insulin sensitivity, lipid metabolism, and energy balance, and dysbiosis is associated with obesity, diabetes, and cardiovascular disease [17,18]. As shown, clinical and preclinical investigations indicate that probiotic strains like *Lactobacillus acidophilus* or *Bifidobacterium lactis* decrease low-density lipoprotein cholesterol and elevate high-density lipoprotein cholesterol levels, lowering the risk of atherosclerosis [17]. Furthermore, probiotics adjust the hormones that control appetite, such as leptin and growth hormone, which lead to weight management and metabolic health [18].

Although there is substantial research evidence of the health-promoting benefits of probiotics, the results of using probiotics in therapy are highly heterogeneous and quite erratic in different clinical circumstances. Most traditional probiotic strains have a low survival at gastric acidity and bile salt conditions, which reduces intestinal viability and leads to poor colonization of the intestinal tract [19,20]. Moreover, the efficacy of probiotics is inextricably strain-specific and highly determined by host supplement body of microbiota, diet, and immune cosmology with unforeseeable consequences for different people [21]. Even though formulation-based strategies have been invented, including microencapsulation and nano-coating, to improve the delivery of probiotics, these methods failed to resolve inherent biological limitations to stress tolerance, adhesion effectiveness, metabolic production, and functional stability. Taken together, these constraints point to the necessity of implementing strategy-based means of precision, which would allow positively impacting the genes and functions of probiotics to enable genome editing and probiotic engineering by synthetic biology.

Synthetic biology combines with genome-editing technology to enable the development of smart programmable microbes that solve medical complications in new ways. Ongoing research and regulatory procedures will enable genetically modified probiotics to transform modern medicine through advanced clinical solutions for diseases and better stomach health, immune system performance, and total wellness.

3. GENOME SHUFFLING APPROACH FOR ENHANCEMENT OF PROBIOTIC EFFICIENCY

The constraints of traditional probiotic preparations have led to the necessity to switch from phenotypic strain selection to genetic-based intervention. Innovative strategies in the early probiotic enhancement procedures used adaptive evolution, encapsulation, and random mutagenesis in order to enhance survival and functionality. Although these methods were producing soft improvement, they were not precise and tended to produce unstable phenotypes or unwanted genetic modifications [30,31]. As a result, the advent of genome editing and synthetic biology has been a paradigm shift in probiotic studies, allowing control of probiotic properties rationally and specifically on the basis of achieving therapeutic effect (Fig. 2).

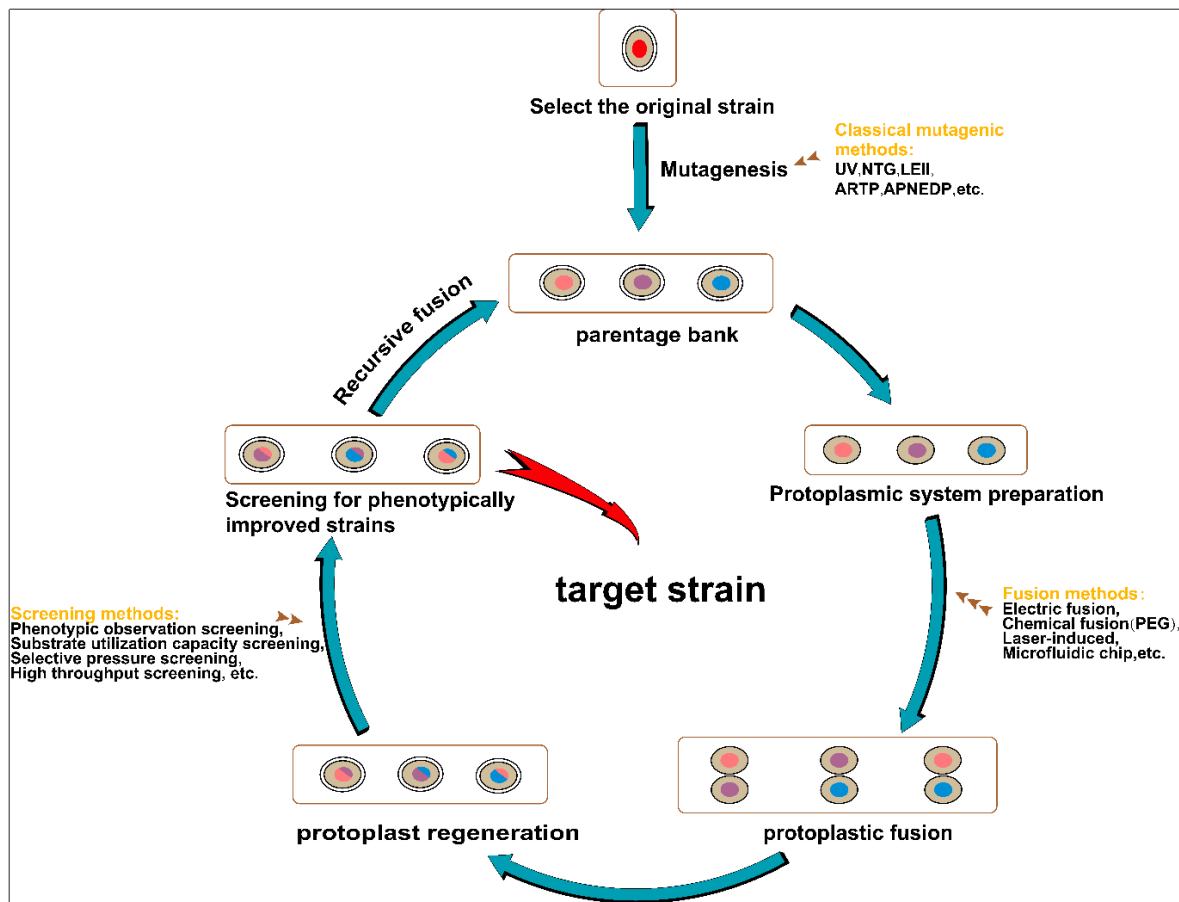


Figure 2. Improvement of bacterial strains using genome shuffling. This workflow consists of selecting parental strains, fragmenting and recombining the genome, reassembling the population by recombination, selecting a better variant, and repeating rounds of genome shuffling. Adapted from Ref. [27].

Genome-editing technologies give the capability to alter a set of genetic loci that encode acid resistance, bile tolerance, adhesion, immune modulation, and bioactive metabolite synthesis. In contrast to the approaches based on mutagenesis in the past, the contemporary genome-editing technique allows predictable, reproducible, and stable improvements of probiotic activity [24]. These innovations have placed probiotics as active dietary supplements against passive biotherapeutic agents and engineered live biotherapeutic agents with established mechanisms of action.

3.1. CRISPR–CAS Systems: Precision and Versatility

The ability to be programmed and efficiency and scaling make CRISPR-Cas systems the most commonly used type of genome editing in probiotic engineering. CRISPR-Cas9 provides the ability to delete, insert, and regulate genes, making it very easy to carefully alter probiotic genomes to provide improved production of gastric and mucosal properties and growth of metabolites [26]. Disruption of metabolic bottlenecks with the help of CRISPR has been revealed to considerably increase the levels of short-chain fatty acids (SCFAs), vitamins, and immunomodulatory molecules in *Lactobacillus* and *Bifidobacterium* strains [32].

In addition to Cas9, more recently discovered CRISPR variants have provided greater flexibility of functionality. Cas12-based systems have made it achievable to target via alternative approaches with significantly less off-target effects, whereas Cas13 provides

ribonucleic acid (RNA)-level regulation without non-reversible genomic editing, providing reversible control of gene expression [33]. CRISPR interference (CRISPRi) has also made probiotic fermentation and storage by transcriptional silencing of stress-sensitive genes much safer [34]. All these methods show that CRISPR systems are most effective in engraining specific traits and building programmable probiotics, especially in cases that necessitate regulatory management and resemblance.

Yet, CRISPR-based editing is not without limitations. Cases of cleaving the target off-target, reliance on protospacer-adjacent motifs (PAM) sequences, and the issue of horizontal gene transfer are and will continue to be technical and regulatory problems [35]. These limitations demonstrate the necessity of complementary or alternative techniques of genome engineering.

3.2. Genome Shuffling: Rapid Multi-Trait Optimization

Genome shuffling is an entirely new approach to probiotic improvement, where recombination-mediated evolution is used, instead of site-specific editing. Genome shuffling allows mutual concomitant enhancement of multiple and polygenic traits including stress resistance, adhesion, antimicrobial activity, and metabolic efficiency by inducing massive genomic recombination within the selected parental strains [27,36].

Research conducted with regard to *L. plantarum* and *Pediococcus acidilactici* has been proposed as candidates for improved intestinal

Table 1. Comparative evaluation of genome-editing technologies in probiotic engineering.

Technology	Precision	Multitrait optimization	Scalability	Regulatory readiness	Clinical maturity	Best-suited application
CRISPR–Cas	High	Moderate	High	Moderate	Preclinical	Therapeutic and programmable probiotics
Genome shuffling	Low	High	High	Moderate–Low	Preclinical/industrial	Industrial strain improvement
ZFNs	Very high	Low	Low	Low	Conceptual	Targeted niche applications
TALENs	Very high	Low	Low	Low	Conceptual	PAM-independent editing
Base editing	Very high	Low	Moderate	Emerging	Early-stage/preclinical	Fine metabolic tuning
Prime editing	Very high	Low	Low–Moderate	Emerging	Early-stage/preclinical	Precise therapeutic engineering

Technology ratings are not a validated quantitative scoring system but rather a qualitative assessment and synthesis drawn from literature sources. The term "clinical maturity" is used in reference to the evidence currently available for engineered probiotics, where "conceptual" indicates a primarily theoretical or extrapolated role, "preclinical" indicates that the evidence is based on in vitro or animal studies, and "early-stage" indicates experimental early development of engineered probiotics. There are no existing technologies at this time with strong evidence for this technology in the engineered probiotic form.

adhesion and colonization following genome shuffling, though the primary evidence specific to shuffled (rather than conventionally supplemented) strains of these species remains limited [37,38]. Additionally, shuffled lactic acid bacteria have been found to produce more antimicrobial peptides and be more effective in pathogen inhibition than parent strains [39]. Improved acid and bile resistance following genome shuffling has also been reported in some lactic acid bacteria, although reliable assessment of such gains depends on methods for accurately measuring probiotic viability under simulated gastrointestinal conditions, an area that itself remains methodologically challenging [40].

Genome shuffling should not be equated with precision genome editing: it is fundamentally a recombination-based strain-improvement strategy, closer in principle to accelerated adaptive laboratory evolution than to site-specific tools such as CRISPR–Cas, ZFNs, TALENs, or base and prime editing. Compared with classical random mutagenesis, genome shuffling recombines whole parental genomes rather than introducing point mutations, which can accelerate the accumulation of beneficial polygenic traits, but compared with adaptive laboratory evolution it does not necessarily improve control over the pace or direction of genetic change. In all three approaches, the causal genetic basis of an improved phenotype is difficult to pinpoint, which complicates mechanistic validation. Genome shuffling shares several practical limitations with these other non-targeted approaches: genetic instability over successive generations, poor reproducibility between shuffling rounds and laboratories, and difficulty attributing a specific functional improvement to a defined genetic change. Because the resulting strains typically retain an undefined pattern of genomic recombination rather than a characterized, intentional edit, genome-shuffled probiotics are generally more readily accepted for industrial or food-grade strain improvement than as candidates for regulated therapeutic development, where regulators typically expect a fully characterized genetic modification.

3.3. ZFNs and TALENs: High Specificity, Limited Scalability

Some of the first programmable genome-editing tools were ZFNs and TALENs; these technologies are still relevant in practice in the niche of probiotic engineering. The high sequence selectivity and lack of PAM requirements provide these systems with the ability to reach genomic areas that cannot be edited using the CRISPR system [41].

TALEN-mediated editing has been proposed as a route to improve stress and metabolic capacity in probiotic strains, including higher butyrate yields through optimization of the relevant pathway,

although this remains a conceptual application rather than a directly demonstrated one [42]. Yet, the design of ZFNs and TALENs is so complicated, costly, and labor-intensive that it limits both their scale and application to large scale. Therefore, these technologies have been shown to be most applicable in high-precision, low-throughput applications, especially where the use of CRISPR-based methods is not possible.

3.4. Base and Prime Editing: Next-Generation Precision Tools

Base and prime editing are some of the key innovations in genome engineering, which allow making specific nucleotide replacements without breaking the DNA strands. Single-base conversions are made possible by the use of base editors, and targeted insertions, deletions, and substitutions with as little genomic interference as possible can be achieved through prime editing [43]. Such tools have been proposed, by analogy with their use in other organisms, as a prospective route to optimizing probiotic metabolism, increasing SCFA production, and boosting stress response, although direct demonstration in engineered probiotic strains remains largely conceptual at this stage [44,45].

The major benefit of base and prime editing as compared to other mutagenic agents is that these two methods have less likelihood of causing unwanted mutations and genomic instability, and they are especially considered desirable in the development of therapeutic probiotics. Nevertheless, there are difficulties with the efficiency of delivery, editing range, and cost, which prevents shot use at scale at present [46]. These technologies are currently best applied to fine-tuning essential functional genes as opposed to general strain optimization. The optimal technology of genome editing based on probiotic engineering does not exist. Precision applications are dominated by CRISPR-based systems, genome shuffling is good at multitrait robustness, and base and prime editing provide an unmatched degree of accuracy in the fine-tuning of therapeutics. These tools are best integrated strategically rather than used in isolation, as dependence on a single tool would limit progress; a comparative evaluation of the five technologies against the five criteria used in this review is summarized in Table 1.

4. TRADITIONAL APPROACHES VERSUS MODERN GENOME-EDITING TECHNOLOGIES

The conventional method to enhance probiotics by random mutagenesis and selection involves exposure of bacterial strains to ultraviolet light or even to chemical agents that induce mutations. The essence of the experiments is to discover more favorable characteristics that

enhance probiotic abilities such as enhanced gastrointestinal tract survivability and enhanced antimicrobial properties. The mutagenesis process creates an excessive number of random mutations through which only a minimal fraction leads to beneficial changes [31]. The deficiency in precision during this process results in difficulties in achieving consistent production of strains with specific characteristics. Labor-intensive and time-consuming remains the nature of selection processes because they depend on phenotypic screening.

5. LIMITATIONS OF CONVENTIONAL PROBIOTIC ENHANCEMENT

Traditional probiotic enhancement faces limitations because each isolate demonstrates specific outcomes. The numerous genetic types within probiotic bacteria create a hindrance because beneficial mutations in some strains cannot transfer to different strains. The traditional probiotic engineering methods experience scalability restrictions because of this limitation [30]. Enhanced strains demonstrate difficulty when it comes to maintaining stability. Through traditional probiotic methods, bacterial changes can cause genetic instability, which leads to a decrease in the targeted beneficial trait after repeated bacterial growth periods. Commercial applications face major challenges from this issue because manufacturers need probiotics to maintain beneficial features while being stored for use and throughout ingestion [47].

The process of traditional probiotic enhancement faces regulatory obstacles that create substantial hurdles. Current prokaryotic strains developed by random mutagenesis procedures are exposed to restrictive genetically modified organism (GMO) guidelines established by numerous national regulatory bodies. Traditional mutagenesis methods that avoid foreign DNA insertion face regulatory barriers similar to genetically modified microorganisms; thus, it becomes challenging to market these strains according to [48]. The imprecise nature of genetic modifications creates additional challenges during regulatory review because parties responsible for evaluations need to perform extensive safety and efficacy tests to prove the absence of unanticipated effects.

The modern genome-editing approaches provide researchers with an efficient and targeted method to enhance probiotics. The CRISPR-Cas9 system provides researchers with an accurate method to modify specific genome positions, which helps them add or delete genes [49]. The revolution in probiotic research has become possible through this technique since it enables scientists to create probiotic strains that demonstrate better acid resistance, improved cell adhesion, and elevated beneficial metabolite production. The precision of CRISPR-based editing surpasses traditional approaches because it applies purposeful changes that lead to enhanced reliability and stability.

Modern site-specific genome-editing techniques with recombination methods help boost probiotic functionalities. These modern molecular tools allow beneficial gene additions through approaches that exclude DNA foreign insertion, thus resolving GMO regulation issues [50]. Scientists improve the antimicrobial peptide production of *Lactobacillus* strains through recombineering strategies in order to achieve better intestinal pathogen competition. Synthetic biology strategies in metabolic engineering resulted in new probiotic bacterial strains designed to create therapeutic substances suitable for treating IBD with anti-inflammatory compounds [51].

The field of probiotic research experienced further growth because of the applications of high-throughput omics technologies. Research strategies using metagenomics, transcriptomics, and proteomics provide detailed information about probiotic-host communication, enabling scientists to create better functional probiotic strains [52]. Sequencing multiple layers of genomic data has led researchers to seek

genetic elements that determine how probiotic bacteria work so they can create specific changes to the bacterial genome. The development of improved health benefits in next-generation probiotics became possible through precise engineering approaches that produced probiotic strains able to affect the gut-brain axis to enhance mental wellness [53].

The contemporary genetic editing methods provide improved advantages when compared to conventional methods yet maintain several important obstacles. The main limitation of genome-editing techniques is their ability to cause unintended genetic changes. Research teams continue to study ways of boosting CRISPR precision delivery because current strategies still need improvement. Gained public acceptance and regulatory requirements continue to shape how genetically edited probiotics are received by people. To become commercially available, genome-edited strains need to pass thorough safety assessments because their long history of safe use is limited to traditional probiotics [35]. Ongoing research about the safety and efficacy of genome-edited probiotics gradually creates acceptance among various parties.

Research on probiotic engineering now explores the creation of synthetic microbial consortia that allow different engineered strains to team up and improve gut health effectively. Researchers at present create microbial communities composed of strains that complement each other to achieve improved nutritive metabolism and pathogen protection abilities. By uniting classical strain selection methods with advanced genome-editing techniques, the strategy provides a proportionate method for enhancing probiotics [54].

The continuous advancement of research will make the combination of AI and machine learning a vital element for probiotic advancement. The use of AI models enables predictions about genetic modification impacts on probiotic functionality, which speeds up the creation of optimized strains. Scientists use experimental genome-editing techniques with computational tools to develop specific health condition-targeted designer probiotics for treating obesity and diabetes and neurological disorders [55].

Current traditional probiotic improvement techniques provided important starting points, but their inefficiency and instability, together with their regulatory challenges, have slowed down research progress. The precise genome-editing capabilities of CRISPR-based techniques and other modern technological solutions provide solutions that address previous restrictions. Synthetic biology, together with omics technologies and AI-driven probiotic design approaches, makes the future of probiotic engineering promise effective personalized medical solutions for human health.

5.1. Transition to Targeted Genetic Modifications

Targeted genetic modifications in probiotic research adopt a new approach for strain optimization by using precision genome-editing techniques with HTS to develop stronger probiotics for therapeutic applications. Strain improvement of probiotics used to depend on random mutagenesis and selective breeding until genetic engineers applied CRISPR-Cas and genome-editing tools to create precise modifications that boost survival rates, metabolic activity, and host communication abilities [56]. Genetic precision interventions by scientists allow them to both add advantageous qualities while removing unwanted characteristics, which leads to better probiotic performance across human healthcare and industrial domains.

The main benefit of using precision genome editing involves developing optimized probiotics which demonstrate improved

functionality. Scientific teams employ CRISPR-Cas9 and base-editing methods to modify lactic acid bacteria, making them better adapted for intestinal passage and helping them produce active metabolites [27]. Scientists use adaptive laboratory evolution methods together with genome-editing technology for developing microorganisms with enhanced properties of mucosal binding and bile salt resistance [57]. Engineer-designed probiotics now target diseases such as IBD and metabolic disorders through genetic engineering of gut microbiome metabolic paths [58].

The process of selecting better probiotic strains depends heavily on HTS after genome editing. The combination of automated systems with omics-based screening platforms allows researchers to evaluate thousands of mutant strains simultaneously for improved metabolic functions, immune modulation, and industrial fermentation resistance [59]. The use of high-throughput transcriptomic and proteomic analysis methods enables researchers to obtain vital regulatory genes that enable optimization of probiotic host-microbiome collaboration [60]. HTS platforms equipped with machine learning algorithms perform predictive modeling to provide decisions in genome editing, thereby shortening the process of strain development and finding the best genetic arrangements [61]. Researchers have made progress with multiplex genome engineering as a technique that allows multiple genetic loci in probiotic strains to be modified simultaneously. Experts applied this technique to *Bifidobacterium* and *Lactobacillus* species through engineering successful strains that enhance both mucin degradation and short-chain fatty acids and antimicrobial peptides production [61]. The modified probiotics gain improved functionality and market superiority through such enhancements, which help them dominate gut environments while fighting harmful pathogens. The construction of probiotics that can generate therapeutic molecules in the gut became possible through the use of CRISPR-based gene circuits [62].

Several issues still block the successful implementation of genetically modified probiotics because of stability and safety concerns. Genetically engineered probiotics need broad clinical adoption, but regulatory barriers together with public sentiment challenges must be solved. Modern research on biocontainment strategies through kill-switch mechanisms and targeted gene deletions enhances the safety characteristics of engineered probiotics [63]. Host-mediated gene engineering methods are currently investigated to enhance microbiome-host relationships while working independently of modification in probiotic strains themselves [62].

Precision genome editing together with HTS and synthetic biology develops entirely new avenues for investigational research into probiotics. Strains of probiotics with customized functional capabilities demonstrate substantial potential applications in individualized medicine and industrial production and functional nutritional product development. Future developments in these technologies promise advanced health benefits together with biotechnological applications that will establish a new period in microbiome-based therapy.

5.2. CRISPR-Cas Systems in Probiotic Engineering

Genetic engineering has been substantially advanced by CRISPR-Cas systems, which provide exact tools for changing microbial genomic sequences (Fig. 3). These systems in probiotic engineering make possible specific gene modifications that optimize metabolism while increasing stability and enhancing microbe-host relations. This scientific revolution shares its name with CRISPR-Cas9, which provides researchers with new

levels of genomic precision. Scientists have successfully applied CRISPR technology to enhance the properties of *Lactobacillus* and *Bifidobacterium*, which function as main bacteria in gut health management and functional food development. Research teams achieved two essential improvements to metabolite production and gastric survival through targeted gene disruptions of metabolic pathway bottlenecks [32].

Engineers have extended the CRISPR toolbox beyond Cas9 to include Cas12 and Cas13 for probiotic engineering applications. Cas12 enzymes primarily target double-stranded DNA and, once activated, exhibit collateral single-stranded DNA cleavage, a property that has been exploited for diagnostic and DNA-targeted applications rather than for direct RNA regulation. Cas13, in contrast, is the RNA-targeting CRISPR system: it binds and cleaves RNA directly, enabling RNA-interference-like knockdown of gene expression in probiotic bacteria without altering the underlying genome sequence [33]. The method provides essential benefits to maintain bacterial viability throughout storage and will preserve their functionality when ingested. The CRISPRi technology opened new possibilities for these systems, which allow scientists to silence genes without making permanent changes to the genome. CRISPRi constitutes a breakthrough that enables the inhibition of stress-reactive genes, enhancing the manufacturing viability of probiotic bacteria in fermentation settings [34].

The most critical usage of CRISPR systems with probiotics includes creating bacterial strains resistant to phage infections. Bacteriophage infections create significant manufacturing challenges for probiotics because they cause major financial damages to both dairy industries and pharmaceutical manufacturers. Separately, researchers have engineered a probiotic *Escherichia coli* Nissle 1917 strain carrying a Type I-E CRISPR-Cas3 system to block the transfer of multiple antibiotic-resistance genes into the strain [64]. Engineered probiotic cultures gain manufacturing stability while supporting antibiotic resistance programs because this approach blocks resistance gene transfer between bacteria. CRISPR-based approaches for immune modulation strategies have become a subject of investigation to improve the interactions between probiotics and the host environment. Engineered probiotics possess therapeutic potential for IBD along with other immune-related disorders because scientists have learned to control immunomodulatory gene expression [66]. Synthetic biology methods have enabled CRISPR technology to advance more effectively in the development of engineered probiotics. Synthetic gene circuits, when combined with CRISPR systems, enable the development of probiotics that adjust their functions based on detected environmental signals. The engineered *Lactobacillus* strains utilizing CRISPR-based biosensors become pathogenic bacteria detection systems within the gut while also serving as neutralization agents for improved gut health maintenance [67]. The developed strains demonstrate potential for precise probiotic development because scientists can create customized bacterial consortia that optimize their benefits to individual microbiomes.

CRISPR technology enhances the metabolic efficiency of probiotics through its critical applications. Scientists use prime editing, which extends the capabilities of CRISPR to modify probiotic strains by making exact nucleotide sequence modifications through methods that prevent destructive double-strand breaks. The optimization of metabolic paths used for bioactive compound production, specifically short-chain fatty acids and vitamins, benefits the host [68]. Research applications of CRISPR-based knock-out-engineered metabolic flux show great promise for functional food advancement and individual nutritional needs.

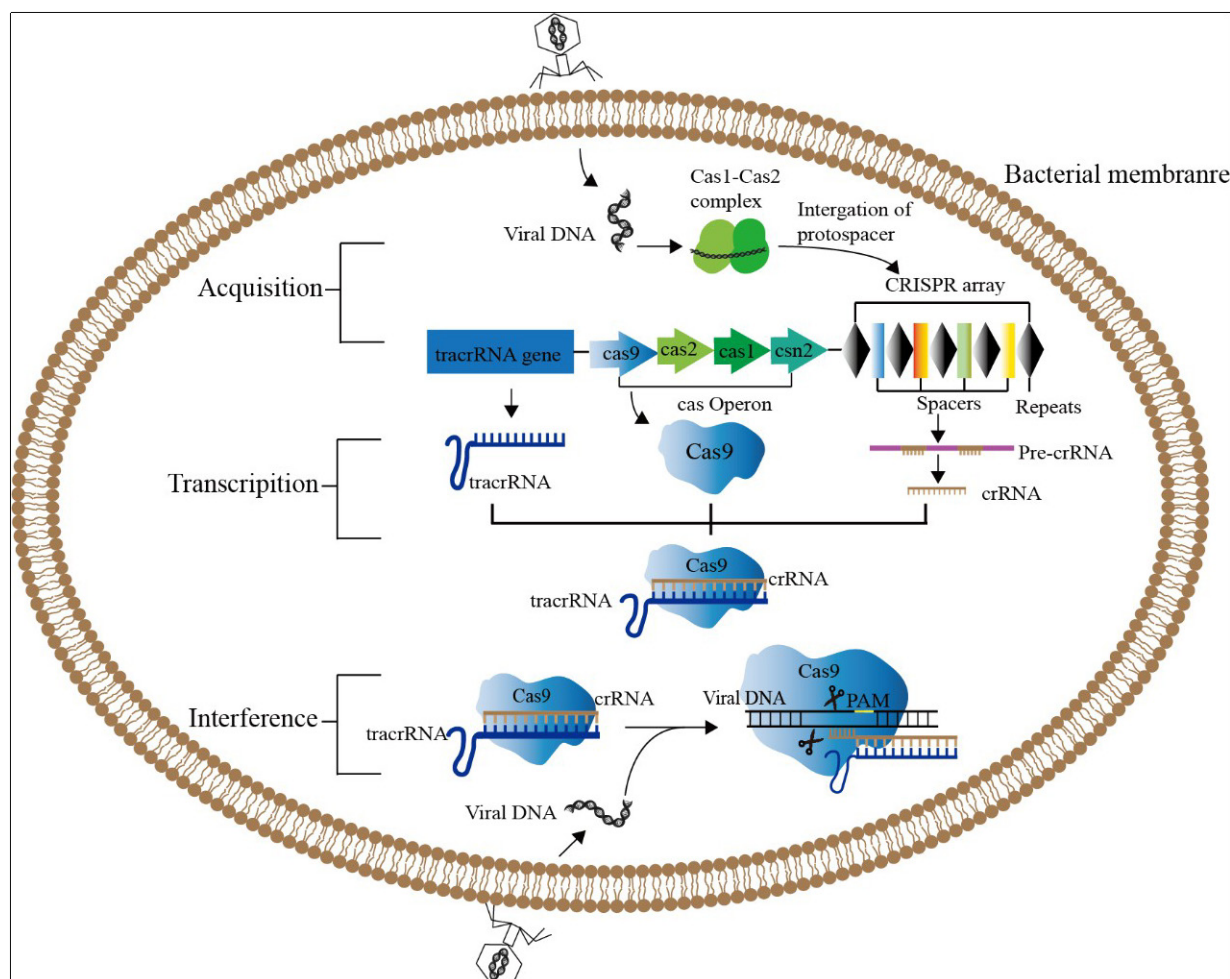


Figure 3. Illustrated mechanism of CRISPR Cas genome editing in bacterial cells. The diagram shows the steps by which the Cas protein guides targeting to the bacterial genome; once the DNA is targeted, the Cas nuclease cuts it, and the resulting DNA repair generates the desired genetic modification. Adapted from Ref. [65].

Researchers have used CRISPR technology to enhance the adhesive abilities as well as colonization performance of probiotics inside the gut. Engineered probiotics survive longer within the intestinal tract because their genetic modification increases binding to mucin and biofilm formation abilities [69]. The next advancement of probiotics requires improved models to fight dysbiosis and rebalance gut microbiota when treating diseases.

Research in probiotics continues to accelerate because of the management capabilities between CRISPR and omics technologies. Modern transcriptomic and metagenomic research has delivered detailed information about how probiotics communicate with hosts, which allows scientists to focus CRISPR interventions effectively. The use of HTS systems enables researchers to find genes suitable for CRISPR modification at exceptional speeds that help speed up the production of improved probiotic medicine [50].

Applying CRISPR-Cas systems to probiotic species also confronts practical barriers that are less prominent in model organisms such as *E. coli* K-12. Many *Lactobacillus*, *Lactocaseibacillus*, and *Bifidobacterium* strains have low natural transformation efficiency, species- or strain-specific codon usage and restriction-modification systems that degrade incoming foreign DNA, and native CRISPR-Cas or other defense systems that can be triggered by the delivered editing machinery itself. Guide RNA and donor-template delivery

is further constrained by limited availability of validated shuttle vectors and origins of replication across genera, and by variable PAM availability in AT-rich or GC-rich genomes. Cas9 toxicity from prolonged expression or leaky nuclease activity has also been reported to reduce transformation yields in some strains. These barriers mean that a CRISPR protocol validated in one probiotic species often needs substantial re-optimization before it can be applied to another.

Because these barriers can produce unintended outcomes, edited strains require systematic post-editing verification before any functional or safety claim is made. Whole-genome sequencing is used to confirm the intended edit and to screen for off-target changes; targeted or genome-wide off-target analysis (e.g., by predicted-site sequencing or unbiased methods) is used to assess specificity; plasmid-clearance assays confirm that any editing plasmid or antibiotic-resistance marker used during construction has been removed; and phenotypic stability testing over serial passage confirms that the edited trait persists rather than reverting. Where CRISPR components (e.g., Cas9 or guide-RNA expression cassettes) are retained in the final strain rather than cured after editing, this itself raises a distinct biosafety consideration—including the possibility of continued nuclease activity, immunogenicity of the Cas protein, or transfer of the CRISPR construct to other microbes—that should be assessed separately from the safety of the intended edit.

The implementation of CRISPR-based probiotic engineering depends heavily on proper regulatory evaluation. Obtaining human consumption approval for genetically modified probiotics requires conducting stringent safety examinations. The evaluation process conducted by regulatory bodies examines both advantages and disadvantages of CRISPR-edited probiotics with emphasis on off-target effects together with horizontal gene transfer risks. The path of CRISPR applications in probiotic engineering demonstrates promising indications for precision-designed probiotics to become vital elements of future personalized medicine, functional nutrition, and gut microbiome modulation.

6. EMERGING GENOME-EDITING TOOLS BEYOND CRISPR

The use of genome editing as a biotechnological tool offers precise genome modification capabilities for probiotics, which has transformed the field. The genome-editing field has seen a rise in popularity for alternative methods such as ZFNs and TALENs during recent years, which demonstrate specific value in improving probiotic robustness [70]. These antique genetic engineering instruments maintain ongoing value because they enable specific gene alterations and function beyond the CRISPR PAM sequence limitation. Researchers now use ZFNs and TALENs as an approach to enhance probiotic survival while maintaining functionality and facilitating stress resistance due to advancements in probiotic genetic knowledge.

6.1. Zinc-Finger Nucleases and Transcription Activator-Like Effector Nucleases

ZFNs introduced the earliest programmable nuclease technology that scientists used for genome engineering. The DNA-binding domain with zinc fingers, along with the *FokI* nuclease domain, enables these components to generate double-strand breaks in desired target loci, according to Saraswat *et al.* [71]. The zinc-finger domains of ZFNs serve to determine recognition sequences that consist of three nucleotide triplets. Multiple zinc-finger motifs combined into assemblies enable researchers to engineer ZFNs able to identify any DNA sequence, thus becoming useful genome-editing instruments for engineered microorganisms [41]. The specific nature of ZFNs plus their ability to function without PAM sequences makes them ideal for modifying probiotic genetics at genomic sites where CRISPR may fail to work effectively. The widespread use of ZFNs remains restricted because their design process is time-consuming and difficult, while their target accuracy poses challenges to researchers.

The development of TALENs brought better modularity and enhanced design capacity compared to ZFNs. The *FokI* nuclease is coupled with transcription activator-like effectors (TALEs), and each TALE domain specifically detects single nucleotides instead of triplets [71]. TALENs offer top-notch sequence selectivity through this design feature that allows them to target different regions encompassing probiotic genetic material. TALENs do not need PAM sequence requirements for operation, so they become effective for genome regions that CRISPR approaches cannot access. Precise genetic alterations in probiotics benefit greatly from TALEN enzymes because their reduced off-target effects surpass those of ZFNs.

Applying ZFNs and TALENs to probiotics serves to increase their resistance against demanding environmental elements. Genetic modification of probiotic strains for food and pharmaceutical usage requires these organisms to withstand processing requirements, including heat exposure and acid and oxidative stress. Genome-editing technologies enabled researchers to develop improved stress-tolerant

strains through gene modifications of protective systems, including heat shock proteins and response pathways toward oxidative stress and acid resistance systems. The genetic loci receive exact modifications through ZFNs and TALENs systems that lead to stable expression of desirable traits because these advancements prevent random genetic changes that occur from traditional mutagenesis methods.

Such genome-editing tools prove essential for the development of improved probiotics through metabolic functionality improvements. Probiotic organisms offer various health advantages due to their biological compound production, which includes antimicrobial peptides, short-chain fatty acids, and vitamins. Researchers have improved the therapeutic value of probiotic strains through TALEN-assisted modification of specific metabolic pathways for increased beneficial metabolite production. TALEN-mediated gene knock-in modifications in *Lactobacillus* and *Bifidobacterium* species have been proposed as a route to enhanced butyrate production, which is important for gut health, drawing on the TALE-repeat design principles described by Rinaldi *et al.* [42], although a direct demonstration of TALEN-edited probiotic strains with increased butyrate output has not, to our knowledge, been reported in the peer-reviewed literature, and this application remains conceptual. The development of customized probiotics for different health applications has the potential to be realized through these functional enhancements.

ZFNs and TALENs have various benefits, yet numerous hurdles restrict their general use in laboratories. The approach to designing ZFNs and TALENs requires long periods of labor and results in complex procedures. The requirement for guide RNAs in CRISPR systems stands in contrast to ZFNs and TALENs because they need extensive sequences to initiate protein-DNA binding operations [72]. Synthesizing and validating ZFN and TALEN constructs proves costly since it makes these technologies less affordable than CRISPR-based systems. Advanced bioinformatics along with synthetic biology enables the creation of improved assembly frameworks that overcome time and expenditure obstacles that used to hinder these technologies.

Genome-editing technology selection depends greatly on the occurrence of unintended effects that happen outside the target region. The enhanced specificity of TALENs surpasses ZFNs, yet the systems continue to generate sporadic unintended cleavage that triggers genome instability. Research into improving TALE repeat domains alongside minimizing *FokI* dimerization needs has yielded better-targeted TALENs that provide stronger reliability in probiotic usages [42]. Research now uses modified *FokI* nucleases with elevated fidelity as a tool to boost the precision of TALEN-mediated editing.

Research into ZFNs, TALENs, and CRISPR demonstrates how these technologies perform in different aspects when used for probiotic engineering. CRISPR technology delivers efficient genome editing through its straightforward process yet requires PAM, recognition points that must follow the target site for Cas9 binding and cleavage but may create critical constraints and possible genomic errors in specific genome structures. ZFNs together with TALENs possess better flexibility in target site selection properties while demonstrating better precision in specific applications. The complex nature of their design and their reduced efficiency in massive editing operations represent major negative elements of these tools. A combination of various genome-editing systems represents the most efficient method for building stronger probiotic strains with improved functions.

The advancement of AI and machine learning algorithms will likely lead to improved ZFN and TALEN design efficiency, which will shorten development periods for probiotic genome engineering

techniques [73]. Until modern, the development of base-editing together with prime-editing methods has brought us new powerful tools to precisely substitute nucleotides without creating double-strand breaks, which has expanded our options for modifying probiotics. Improved probiotic strain modifications are achievable through recent advancements that enhance survival capacity, therapeutic efficiency, and metabolic output potential, leading to customized future microbe-based therapeutic agents.

The pre-eminence of CRISPR in genome editing throughout recent years does not eliminate the significance of ZFNs and TALENs as alternative engineering tools for probiotics, especially when CRISPR shows its limitations in relevant applications [73]. Genetic locus modification through these tools becomes vital for developing robust metabolically enhanced probiotics because they work beyond PAM limitations. These genome-editing technologies progressively improve in technology yet continue serving as essential components to advance microbial biotechnology for health benefits and industrial uses.

6.2. Base Editing and Prime Editing: Next-Generation Precision Tools

Genome editing represents a revolutionary instrument for molecular biologists because it expands genetic engineering prospects that surpass CRISPR-Cas9 technology. Scientists now achieve level-perfect genome modification through base editing and prime editing that lets them modify individual nucleotides without DSBs to decrease genomic instability and unwanted mutations. Recent generation weapons display notable promise when employed in agricultural as well as medical applications through improved probiotic metabolism and targeted human gene function enhancement. Research development enables genome editing to expand with specialized methods for high-specificity DNA modification and efficient operation.

Base editing techniques discovered by David Liu [74] constitute an advanced modification method that introduces chemical changes to single nucleotides while preventing double-strand break formation, thus providing a more precise solution than standard CRISPR-Cas9 technologies. Cytosine base editors (and adenine base editors) are two main base editing classes that perform C-to-T transitions as well as A-to-G transitions but do not require donor DNA templates or homology-directed repair (HDR) pathways, according to Tan [43]. Base editing has been explored as a therapeutic strategy for monogenic human diseases such as sickle cell disease and cystic fibrosis because of its precise, template-free editing mechanism [44]. By extension, base editing has been proposed as a tool to fine-tune metabolic genes in probiotic bacteria to improve dietary-fiber breakdown and SCFA production, but this remains a largely conceptual extrapolation from the human gene-therapy literature rather than a finding demonstrated directly in engineered probiotic strains. The capacity of base editing to enhance key metabolic genes shows potential for developing better nutritional fermented foods and probiotic supplements, which are key areas of investigation in modern microbiome studies.

The prime editing technique serves as a second revolutionary genome-editing platform that extends precision beyond base editing capabilities. Scientists at Anzalone *et al.* [75] created prime editing through a combination of Cas9 nickase with reverse transcriptase fusion and prime editing guide RNA (pegRNA) that works as a director for DNA sequence modification tasks at defined locations [76]. Prime editing differs from conventional CRISPR strategies since it omits both double-stranded breaks and donor DNA templates, thereby becoming a superior option for precise genetic correction of monogenic conditions. Prime editing has been proposed, by analogy with broader gut-microbiota modulation

strategies aimed at metabolic conditions such as obesity, as a potential route to engineering probiotic strains with improved gastrointestinal acid resistance and nutrient handling, although a prime-edited probiotic strain with these properties has not, to our knowledge, been directly reported [45,77]. The recent research shows how engineers can develop modern probiotics with specific health functions that help protect the gut and prevent metabolic diseases.

These genome-editing technologies have shown their most promising use in functional enhancement by avoiding the extensive, uncontrolled genomic disruption associated with double-strand breaks. Standard Cas9-based insertion/deletion editing works by introducing a double-strand break at the target site; in eukaryotic cells, this break is typically resolved by non-homologous end joining (NHEJ) or HDR, but most probiotic bacteria lack an efficient NHEJ pathway and instead rely predominantly on homologous recombination or recombineering (often assisted by phage-derived recombination systems) to repair breaks or incorporate donor DNA, so the NHEJ/HDR framework developed for eukaryotic genome editing does not transfer directly to bacterial hosts. Base editing and prime editing, in contrast, are characteristically double-strand-break-free: they modify or rewrite the target sequence without relying on cellular double-strand-break repair, which is why they are generally associated with a lower risk of unintended indels or larger genomic rearrangements [78].

Beyond probiotic engineering, base and prime editing have an established track record in human gene therapy—for example, correcting variants associated with cardiovascular lipid metabolism [79] and engineering immune cells for cancer immunotherapy [80]—and in agricultural biotechnology, including herbicide-resistant and stress-tolerant crop development [81,82]. These applications illustrate the versatility of the underlying chemistry rather than direct evidence in probiotic hosts; probiotic-specific applications of base and prime editing remain comparatively early-stage by comparison.

Base and prime editing have recorded significant progress, but researchers must resolve technical limitations pertaining to their delivery systems and precision and overall operational effectiveness. *In vivo* applications of base editors face restrictions because their overall editing performance varies depending on the cells and tissue type [46]. The therapeutic usage of prime editing requires further development because a single correction edit is restricted to DNA sequence lengths. The intended improvements in engineered pegRNAs along with new delivery methods and enhanced guide RNA design algorithms will expand the practical usage of these systems [54].

The development of genome editing research brings AI and machine learning into a central position to maximize the potential of base and prime editing strategies. The application of AI-based models enables researchers to predict unwanted effects as well as enhance pegRNA designs and search for optimal editing target sites to boost the accuracy and activity of these techniques [46]. The advancement of genome-editing techniques now merges with epigenetic modifications to create gene control methods that do not change DNA permanently, which holds promise for applications in gene therapy along with regenerative medicine. Genome-editing research shows promise to enter mainstream healthcare applications, which will eventually establish personalized medical treatments for genetic disorder corrections through precise genetic methods.

The combination of base editing and prime editing stands as the future of genome engineering because they outperform CRISPR-Cas9 methods for precise and versatile applications. These editing methods have transformed genetic research because they can modify genes at specific nucleotide levels while avoiding DNA double-strand

breaks that were common among older techniques. Biotechnology and medicine are experiencing transformative changes because these technologies help boost probiotic efficiency, treat genetic conditions, and strengthen plant resistance. Through ongoing research developments, precision genome-editing tools will become more useful because of innovative delivery system discoveries as well as computational modeling advances and epigenetic integration applications that will enable groundbreaking scientific applications across various fields.

6.3. Engineering Probiotics for Enhanced Functional Properties

Scientists have demonstrated increased interest in probiotic engineering for improving functional properties, spanning genetic modification, metabolic engineering, and disease-management applications. This section focuses on two areas central to this effort: engineering probiotics for stronger gut adhesion and colonization, and metabolic engineering for enhanced nutritional and therapeutic output.

6.4. Improving Gut Colonization and Stability

The main difficulty in using probiotics stems from maintaining a stable and established presence of these bacterial cells within the gastrointestinal system. The human gut maintains strong competitive properties, making it difficult for probiotic bacteria to adhere to mucosal surfaces while resisting digestive conditions to form biofilms and persist. Research on genetic engineering solves the key issues that previously stood in the way of progress. Researchers have discovered mucus-binding proteins (MUBs) and fibronectin-binding proteins among other specific surface proteins which demonstrate high potential for use in bacterial adhesion enhancement [83]. Laboratory-modified *Lactobacillus* and *Bifidobacterium* strains that express elevated levels of MUB exhibit better mucosal binding and longer-lived gastrointestinal colonization in animal test subjects [84]. Research has shown that using quorum-sensing circuits creates biofilms that enhance bacterial survival speed and fighting ability toward pathogens [85].

Probiotic stability mainly depends on how well the bacterial strains can withstand stomach acid and intestinal bile salts. The combination of extremely acidic stomach conditions and bile acid presence in the small intestine quickly reduces the viability of probiotics. Acid-resistant bacterial strains have been developed through genetic modification of bacterial proton-pump systems, such as the F1F0-ATPase, and of acid-stress response regulons. Scientists have overexpressed the bile salt hydrolase gene in particular *Lactobacillus* strains to develop their resistance against bile acids, thus enabling them to thrive effectively in the gut environment [86]. Recent advances in probiotic research have improved intervention performance, which leads to stronger gastrointestinal resistance and stronger bacterial establishment within the body.

7. METABOLIC ENGINEERING FOR ENHANCED NUTRITIONAL AND THERAPEUTIC BENEFITS

The process of metabolic engineering has enabled scientists to optimize functions and biological products such as vitamins and enzymes in addition to probiotics. The three short-chain fatty acids of acetate, propionate, and butyrate demonstrate significant importance for gut health because they support beneficial metabolite synthesis while modifying immune responses, maintaining intestinal integrity, and supplying energy to colonic epithelial cells. Modern research shows that *Bifidobacterium* and *Lactobacillus* metabolic pathway modifications result in higher SCFA production levels. Scientific

research has shown that elevating levels of butyryl-CoA:acetate CoA-transferase has brought forth increased butyrate production because this anti-inflammatory compound prevents colorectal cancer [87].

Engineered probiotics now produce fundamental vitamins such as folate, riboflavin, and vitamin B12 through design modifications to help support metabolic functions together with brain-related operations. The modification of *Lactococcus lactis* and *E. coli* Nissle 1917 strains for vitamin biosynthesis has resulted in improved folate and riboflavin content detected through clinical trial analysis [88]. Scientists have used enzymatic modifications to boost probiotic capability for processing dietary substances. The addition of external amylases together with proteases enables complex carbohydrate and protein breakdown, which extends the metabolic benefits for the host according to Flint [89]. Research-based findings demonstrate that probiotics function as bio factories for essential nutrients and will enable their applications in functional foods as well as precision nutrition.

8. PROBIOTIC ENGINEERING FOR DISEASE-SPECIFIC APPLICATIONS

The use of engineered probiotics to manage human diseases becomes more important for treating IBD and IBS and metabolic disorders [90]. The most promising recent development involves creating anti-inflammatory probiotics that regulate immune response functions. Engineered versions of *L. reuteri* and *Bifidobacterium breve* strains have been proposed as a means of producing the anti-inflammatory cytokine IL-10 locally within the gut. Scientific research indicates that these engineered strains of *Lactobacillus reuteri* and *B. breve* contribute to decreased gut inflammation within preclinical colitis and Crohn's disease models, though the specific engineered-strain findings and their attribution warrant source-by-source verification [66,91]. Probiotics that produce antimicrobial peptides including bacteriocins display effective pathogen suppression capabilities, which allows them to restore microbial gut balance [92].

Modern research modifies probiotics to direct metabolic changes in hosts against obesity and diabetes. Genetic tools developed for *Lactobacillus gasseri* [93] provide a platform that has been proposed, though not yet demonstrated in this species, for boosting conjugated linoleic acid production as a strategy to support fat metabolism and reduce adiposity. Conventional (non-engineered) probiotic supplementation has been associated in a systematic review with modest improvements in insulin resistance markers [94]; comparable clinical evidence specifically for genome-edited engineered strains in visceral fat or insulin outcomes is not yet available. Research investigates the use of probiotics to treat patients with non-alcoholic fatty liver disease through gut-liver axis intervention. Research proves that probiotics provide hepatoprotective effects through their ability to control bile acid metabolism as well as their ability to produce SCFAs, which decreases liver lipid accumulation [95]. Probiotics show great potential to function as biotherapeutic agents for treating different chronic diseases through advancements in research.

Across the disease areas discussed above, it is important to be explicit about the maturity of the underlying evidence: with few exceptions, engineered-probiotic applications to IBD, IBS, cancer, metabolic disease, and neurodegenerative or immune disorders remain at the *in vitro* or animal-model stage, and should not be read as validated therapeutic alternatives to existing treatments. Translation to human use also raises patient-specific safety considerations that are distinct from those for conventional probiotics, including the risk

of translocation or bacteremia in immunocompromised patients, uncertain colonization behavior in infants and elderly patients, and altered engraftment in individuals with a disrupted intestinal barrier. Clinical development of engineered strains will additionally need to address dose–response relationships, persistence and shedding of the engineered strain after treatment ends, and confirmation that the strain and any inserted genetic elements can be reliably cleared or contained once therapy is complete.

9. SYNTHETIC BIOLOGY AND PROGRAMMABLE PROBIOTICS

9.1. Synthetic Circuits in Probiotic Control

The resulting cooperation between synthetic biology and probiotic engineering has produced synthetic circuits that precisely manage bacterial activities occurring in human microbiomes [96]. The engineered systems respond programmatically to environmental triggers to create dynamic therapeutic possibilities. Recombinase-based genetic memory circuits, which allow bacteria to record and later report on a transient signal, have been engineered and demonstrated in laboratory *Escherichia coli* strains and are of interest for future integration into probiotic chassis such as *Escherichia coli* Nissle 1917; related, currently available *E. coli* Nissle-based tools include an ingestible biosensor capsule for monitoring gastrointestinal signals and a Type I CRISPR-Cas system engineered to block antibiotic-resistance gene transfer, though neither of these specific tools constitutes a recombinase memory circuit in its own right [64,97]. Conditional genetic switches that couple therapeutic-gene expression to disease-associated biomarkers are an active area of synthetic-biology design and have been demonstrated in principle for other engineered-delivery contexts; extending this concept to reduce off-target therapeutic exposure and unwanted side effects in probiotic chassis remains, to our knowledge, at an early, largely conceptual stage. The utilization of metabolically engineered probiotics forms a core component in 3D-printed bioactive materials. The genetically engineered properties of probiotics contribute to optimization of their functional use in targeted therapy [98]. The ability to precisely control probiotic functions represents a major advancement for precision medicine because doctors can design specific microbiome treatments for each individual patient.

A recurring translational challenge for engineered gut microbes is maintaining circuit function inside a competitive, dynamic gut environment. Synthetic circuits impose a metabolic burden on the host cell, and strains carrying a burdensome circuit are frequently outcompeted by circuit-free revertants under selective pressure over successive generations; plasmid-borne circuits are additionally subject to loss through segregational or structural instability. Robust *in vivo* function therefore depends on chromosomal integration or high-stability plasmid systems, continuous or intermittent selection strategies, and circuit designs tolerant of mutation. Clinical translation of any such circuit will also require containment and reversibility: control over dosage and colonization density, specificity of the input signal to avoid off-target activation, mechanisms to prevent or limit therapeutic-payload leakage, and, ideally, a means to eliminate the engineered strain after treatment if required.

9.2. Programmable Probiotics in Personalized Medicine

The integration of biosensors inside probiotics enough consumers with a groundbreaking system to track gut microbiome dynamics and deliver targeted therapeutic interventions. The engineered bacteria contain genetic circuits that detect selective metabolites and inflammatory

indicators or pathogenic bacteria to assess sufferers' gut health status. Research shows that innovative biosensors effectively measure colonic inflammation, which assists medical staff in detecting IBD during its early stages [97]. According to quorum-sensing principles, these sensors generate signals by measuring alterations in microbial communities in order to activate quick therapeutic responses. The selective elimination of pathogenic bacteria through CRISPR-based gene editing systems in probiotics allows for establishing microbial balance [64]. Biosensor-integrated probiotics have been proposed as a tool to improve drug pharmacokinetic monitoring through oral administration, which secures adequate drug therapy while minimizing negative side effects. Modern research in gut microbiome management has expanded the potential use of probiotics as combined diagnostic and therapeutic tools that can treat conditions actively through genetic editing [99].

Artificial intelligence is being explored as a tool to help design probiotics suited to different microbiome profiles. Machine-learning models trained on microbiome datasets have been used to help identify candidate strains and predict community-level interactions, and computationally guided design of synthetic bacterial consortia has been proposed as a strategy to improve microbial diversity in metabolic and gastrointestinal dysbiosis [100]. These AI-based approaches depend on the quality and representativeness of the underlying training data and generally still require experimental confirmation of predicted strain interactions; claims about fully individualized, AI-designed probiotics remain aspirational rather than clinically established. The integration of AI systems helps scientists identify how different microorganisms will interact within host environments to maintain proper function of engineered probiotics. The evidence confirms this methodology helps create individualized probiotics that enhance digestion and absorb nutrients by using specific genetic and microbial data of each person [101]. The advancement of AI technology will drive synthetic biology research toward future-generation probiotics with precise therapeutic functions.

9.3. Precision Probiotics for Cancer Therapy and Chronic Disease Management

Researchers currently recognize programmed probiotics as increasingly important tools for oncology practice and chronic disease management. The laboratory manipulation of bacteria yielded cancer-detecting cells that emit toxic substances targeting cancer tissues after identifying specific cancer-related chemicals [102]. Programmed probiotics now have the ability to control immune system functions, which improves the effectiveness of cancer treatments using immunotherapies. Studies using engineered *Lactobacillus* strains showed promising mouse model results that indicated suppression of tumor development according to Xu *et al.* [103]. Precision probiotics show promise for managing both cancer conditions as well as metabolic disorders, autoimmune diseases, and neurodegenerative diseases. Synthetic biology enables programmable probiotics to redesign disease treatment approaches by providing individualized solutions that exceed pharmaceutical standards.

10. CHALLENGES AND ETHICAL CONSIDERATIONS IN PROBIOTIC GENOME EDITING

The primary safety issue about engineered probiotics includes horizontal gene transfer (HGT), which allows external DNA in genetically modified bacteria to exchange with gut microbiome species or environmental microbes. Unintended events from this practice can result in the transfer of antibiotic resistance genes, which creates a major health risk for the public [104,105]. Research indicates that beneficial trait-conveying engineered probiotics can accidentally

provide genetic material to pathogenic bacteria, thus enabling the pathogens to increase their virulence or acquire antimicrobial resistance. The stability of genes inserted into probiotic strains poses challenges because spontaneous mutations together with gene excision events can modify the genetic function, thus causing unpredictable effects on human health [106]. Researchers have developed new CRISPR-based containment methods by developing DNA constructs which trigger host cell death upon unwanted DNA transfers between different hosts [107]. Researchers demand strict risk evaluation methods that should include detailed metagenomic analysis to check for gene transfer occurrences in human microbiota. Prior to market release of genome-edited probiotics, regulatory authorities need to perform extensive safety evaluations to verify that the advantages exceed possible risks that arise from horizontal gene transfer and antibiotic resistance mechanisms.

For clarity, the principal biosafety concerns for engineered probiotics are distinct and are best assessed separately rather than treated as a single “safety” category. Off-target editing refers to unintended sequence changes introduced by the editing tool itself and is assessed by whole-genome sequencing and off-target-site analysis, as noted above. HGT and antimicrobial-resistance marker transfer refer to the movement of genetic material, including any residual selection markers, from the engineered strain to other gut or environmental microbes; this can be experimentally assessed using filter-mating or co-culture conjugation assays, *in vitro* gut-model systems, and metagenomic sequencing of exposed microbial communities to detect transferred sequences, and can be reduced by removing antibiotic-resistance markers, using scarless editing and marker-free selection strategies, and avoiding mobile genetic elements in the final construct. Microbiome disruption refers to unintended shifts in the composition or function of the resident gut community following administration, which is assessed longitudinally with metagenomic or 16S sequencing. Immune activation refers to host inflammatory or immune responses to the engineered strain or its products, assessed via cytokine and immune-cell profiling in preclinical models. Environmental release refers to shedding of the engineered strain into wastewater or the wider environment after excretion, which requires environmental survival and dissemination studies distinct from those addressing safety inside the host.

Genomic and phenotypic stability should be evaluated over serial passages in culture and, where possible, under simulated gastrointestinal conditions (sequential acid, bile, and enzymatic exposure), since an edit or circuit that appears stable after a few generations may still be lost or mutated under the selective pressures of repeated growth. Kill switches and other genetic containment systems are frequently proposed as a safeguard, but their reliability is limited in practice by predictable failure modes: escape mutants that inactivate the kill-switch circuit under selective pressure, metabolic burden that itself drives loss of the containment construct, and incomplete penetrance where a small surviving subpopulation persists. These failure modes mean that a reported kill-switch design should be evaluated on measured escape-mutant frequency and stability over time rather than assumed to provide absolute containment. Finally, biosafety assessment should explicitly address the fate of the engineered strain after it leaves the host, including survival and potential horizontal gene transfer in wastewater and other environmental reservoirs, which is rarely characterized in current probiotic-engineering studies but is relevant to environmental risk assessment.

11. GLOBAL REGULATORY LANDSCAPE FOR GENETICALLY ENGINEERED PROBIOTICS

Different nations follow distinctive methods to evaluate the safety and efficacy aspects of genome-edited probiotics while their regulatory

framework remains in development. The FDA of the United States places genetically modified probiotics within the classification “live biotherapeutic products,” so scientists must conduct thorough clinical assessments to gain permission [104]. Genome-edited probiotics face strict European Union regulations because they receive the same treatment as genetically modified organisms through deep risk evaluations and labelling requirements. The conflicting regulatory systems between different countries present difficulties for researchers and biotech organizations trying to achieve worldwide commercialization because they face greater expenses and longer time durations when they need to pass multiple approval systems [106]. The regulatory systems in Japan and Canada embrace a rational strategy that evaluates genome-edited probiotics through examinations of their modifications instead of analyzing the genetic change methods. The community of experts stresses that international regulators need to unify their standards according to scientific evidence and ethical considerations through a standardized risk assessment system. The absence of regulatory alignment will create problems in innovation, together with constrained technology access for probiotic genome editing, which consequently affects public health benefits.

Rather than being uniformly “strict” or “relaxed,” regulatory treatment differs primarily by product classification, the nature of the genetic modification, and intended use. In the United States, a genetically engineered probiotic intended to treat, cure, or prevent disease is generally regulated by the FDA as a LBP, following a biologics licensing pathway that requires Investigational New Drug clearance and phased clinical trials; a genetically engineered strain marketed for general wellness may instead fall under dietary-supplement or conventional-food frameworks, which carry substantially lighter evidentiary requirements. In the European Union, an engineered strain typically falls under GMO-specific legislation (Directive 2001/18/EC and related regulations) in addition to Novel Food Regulation (EU) 2015/2283 if intended for food use, both of which require a dossier addressing molecular characterization, environmental risk assessment, and labelling. Regulators in several other jurisdictions, including Japan and, for certain classes of modification, Health Canada, apply a product-based rather than a process-based framework, focusing evaluation on the characteristics of the final organism rather than treating every genome-editing method identically; whether a given edit is classified as equivalent to conventional breeding or as a GMO modification can depend on factors such as whether foreign DNA is retained in the final strain.

Across jurisdictions, regulators generally expect dossiers addressing strain identity and genetic characterization (including confirmation of the intended edit and absence of unintended off-target changes), purity, potency and stability of the drug product, safety pharmacology and toxicology data, evidence on shedding and environmental risk, and, for therapeutic indications, clinical efficacy data. Because genome-edited probiotics are a comparatively new product class, post-market surveillance and pharmacovigilance frameworks specific to live biotherapeutics are still being developed in most jurisdictions; the resulting heterogeneity in classification and evidentiary expectations, rather than any single agency being simply more or less permissive, is the principal source of the cross-border delays and additional cost that regulatory heterogeneity imposes on developers.

12. ETHICAL CONSIDERATIONS IN HUMAN MICROBIOME ENGINEERING

Genome editing of probiotics creates fundamental moral issues about how humans should work with their microbiome. The scientific breakthrough of engineered probiotics for disease prevention and treatment remains remarkable, but doubts exist about ecosystem

disturbances and lasting effects on human evolutionary development [108]. Genetic modifications to the gut microbiome can destabilize its natural regulatory mechanisms, which can lead to potential unexpected health threats because this system controls both immune system functions and metabolism. The lack of complete understanding about genetically engineered microbes raises ethical concerns because consumers need clear labelling and proper education [109]. Bioethicists state that before genome-edited probiotics can reach the public for use, researchers need to conduct prolonged preclinical and clinical tests that demonstrate no harm to patients. The technology should provide equal opportunities to all communities to avoid increasing current health gaps between economically different populations. The development of probiotic genome editing needs both extended public participation and multidisciplinary dialogue to fulfil responsible and socially appropriate progress.

13. ADDRESSING MISINFORMATION AND IMPROVING PUBLIC TRUST IN GENOME-EDITED PROBIOTICS

Genetically engineered probiotic acceptance faces strong opposition because the general public has improperly informed opinions about genome-editing techniques. Consumer reluctance stems from misunderstandings about probiotics as dangerous alterations to human DNA and their nature as genetically modified products, even though scientific data proves otherwise [104]. The primary obstacle rests in transmitting correct information about benefits alongside risks of editing probiotic genomes to population groups while using clear, accessible communication channels. The public reliance on government regulatory bodies and scientific institutions determines consumer perceptions about probiotic genome editing, so scientists and administrators must lead active outreach for scientific education. Social media requires strategic use in order to spread factual information while fighting against false scientific ideas [106]. Without ethical labelling practices as well as third-party safety certifications and public stakeholder involvement in regulation-making decisions, trust and acceptance can increase. Open communication between scientific experts and policymakers and consumers will enable evidence-based policy development with ethical factors to guide the responsible implementation of genome-edited probiotics in mainstream healthcare.

14. FUTURE PERSPECTIVES AND TRANSLATIONAL OUTLOOK

Genome editing, systems biology, and microbiome science has radically transformed the field of probiotics to be no longer based on an empirically chosen selection of dietary supplements but a rationally designed and functionally defined live biotherapeutic. The traditional probiotics, despite their utilization, have limitations based on the variability of the strains, lack of gastrointestinal resilience, and uncertainty in the functional results within the population of the host [19–21]. Biotechnologies of genome editing can be used to address these limitations with a strong framework in which probiotic characteristics can be precisely, stably, and reproducibly enhanced to directly change the colonization efficiency, immune modulation, and production of bioactive metabolites [24,25].

It is projected that further developments in probiotics will be focused more on condition-specific and customized use in the future. Multi-omics (encompassing genomics, transcriptomics, proteomics, and metabolomics) should be incorporated in their bid to map the mechanism of probiotic-host interactions centrally; in addition, genetic determinants loyal to therapeutic effect should be determined [28]. These datasets, coupled with AI-based predictive modeling, will

speed up the optimization of strains; predictive targeting of metabolic and disease-specific probiotics will be possible, and custom probiotics based on an individual microbiome composition and disease environment will be differentiated [27].

These technological advances do not mean that genome-edited probiotics can be widely used in clinical settings until a number of translational challenges are overcome. The regulatory landscape of genetically modified microorganisms is disjointed, and biosafety and environmental risk assessment standards are restrictive toward the fast commercialization of these technologies [22]. Problems associated with horizontal gene transfer, long-term host safety, and the impact of gene transfer on ecology also require that genetic restriction techniques such as auxotrophic designs, kill-switches, and non-replicative delivery platforms need to be integrated [26]. These issues will be critical in dealing with regulatory acceptance and popular trust. Scalability of manufacturing and economic viability are also major constraints. Although the CRISPR-Cas systems have high editing efficacies and accuracy, downstream issues encompassing fermentation stability, batch-to-batch reproducibility, and cold-chain logistics remain problematic for industrial-scale applications [34,35]. Genome shuffling offers benefits of increasing multitrait robustness and industrialized resilience at no cost and is not as predictable mechanistically, which makes regulation increasingly difficult to validate and to control quality [27]. Base and prime editing, despite being more accurate than any other technique, are limited in delivery efficiency and cost-effectiveness, which limits their use to specific, high-value therapeutic applications [46].

In the future, techniques in genome-edited probiotics will not only rely on their level of sophistication in the areas of technology but also in terms of clinical relevance, safety, and translational viability. In spite of the fact that engineered strains often show improved functionality *in vitro* and in preclinical models, well-designed, controlled human clinical trials specifically evaluating genome-edited probiotics remain scarce. Long-term effectiveness, safety, and persistence in numerous groups of people should be determined by rigorous randomized, placebo-controlled studies with standardized endpoints. Similar improvements in the area of pharmacovigilance and post-market surveillance will further enhance the translational credibility.

All these advancements together help emphasize that no single genome editing therapy would be the most effective in all cases involving probiotic enhancement. CRISPR-Cas systems are widely utilized in precision engineering; multitrait robustness is optimally met by genome shuffling, and fine-scale metabolic quieting, available to base and prime editing, meet distinct biological and transformational needs [24,27,76]. Such complementary technologies, integrated strategically with the aid of omics-driven insight, regulatory foresight and clinical validation, will finally reveal the next generation of probiotic therapeutics and will define their success in moving beyond laboratory innovation into actual health practice.

Translating any of these technologies from the laboratory to a candidate live biotherapeutic benefits from explicit go/no-go decision points rather than an open-ended development timeline. At the strain-selection stage, a candidate should be deprioritized if it shows unresolved genomic instability over serial passage, an inability to achieve reproducible transformation or editing efficiency, or evidence of retained mobile genetic elements that cannot be removed. At the edit-validation stage, unacceptable off-target edit frequency, failure to clear editing plasmids or antibiotic-resistance markers, or loss of the intended phenotype on gastrointestinal-condition simulation

should trigger a return to strain redesign rather than progression to animal studies. At the preclinical stage, uncontrolled colonization behavior (persistent, unintended overgrowth or, conversely, failure to engraft), demonstrable horizontal gene transfer in gut-model or animal systems, or weak or inconsistent therapeutic output relative to an unedited comparator strain should likewise halt advancement. Finally, at the point of clinical and regulatory planning, a project should be reconsidered if the intended product classification, and the corresponding evidentiary pathway, cannot be clearly established with the relevant regulatory authority in advance. These criteria will necessarily need to be adapted to the specific probiotic species, editing technology, and target trait under development rather than applied as a fixed checklist.

15. CONCLUSION

Genome editing and synthetic biology have opened a genuinely new set of tools for probiotic strain development, but the evidence reviewed here does not support the claim that these tools have already transformed probiotics into validated live therapeutics: with few exceptions, precision-engineered probiotics remain at the preclinical, *in vitro*, or early translational stage, and the clinical evidence available for genome-edited strains specifically—as distinct from conventional probiotic supplementation—remains limited. Clinical translation continues to be constrained by strain- and species-specific editing barriers, the need for standardized safety and stability testing, heterogeneous and still-developing regulatory pathways, delivery and manufacturing scalability, and public acceptance.

No single genome-editing technology is likely to be sufficient on its own: CRISPR-Cas systems currently offer the best-characterized route to precision editing in probiotic hosts, genome shuffling remains useful chiefly for industrial multitrait strain improvement rather than regulated therapeutic development, and base and prime editing, while promising for fine-scale metabolic tuning, remain comparatively early-stage and species-dependent in bacteria. Realizing the potential of precision-engineered probiotics will depend on combining complementary editing technologies with multi-omics and computational tools, standardized safety and stability testing, and clearer, more harmonized regulatory pathways, so that promising laboratory findings can be evaluated through the same rigor of clinical validation expected of any other biological therapeutic.

16. USE OF ARTIFICIAL INTELLIGENCE (AI)-ASSISTED TECHNOLOGY

The authors declare that they have not used artificial intelligence (AI)-tools for writing and editing of the manuscript, and no images were manipulated using AI.

17. CONFLICT OF INTEREST

The authors report no financial or any other conflicts of interest in this work.

18. AUTHOR CONTRIBUTIONS

All authors made substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; took part in drafting the article or revising it critically for important intellectual content; agreed to submit to the current journal; gave final approval of the version to be published; and agree to be accountable for all aspects of the work. All the authors are eligible to be author as per the International Committee of Medical Journal Editors (ICMJE) requirements/guidelines.

19. ETHICAL APPROVALS

This study does not involve experiments on animals or human subjects.

20. DATA AVAILABILITY

All the data is available with the authors and shall be provided upon request.

21. PUBLISHER'S NOTE

All claims expressed in this article are solely those of the authors and do not necessarily represent those of the publisher, the editors and the reviewers. This journal remains neutral with regard to jurisdictional claims in published institutional affiliation.

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