

Recent advances in engineered edible probiotic vaccines: Promising agents in the effectiveness of probiotics

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Abstract

Background and Objective: Associations between probiotics as dietary supplements for health enhancement and illness management and human health include a long history. Currently, probiotics represent a potential category of microorganisms used in the development of oral vaccines for the treatment of allergies, infectious diseases, and cancers. The vaccine promises as safe therapeutic options, their capacity to elicit mucosal and systemic immune responses, and their cost-effectiveness resulting from the absence of complex purification processes have been addressed.

Results and Conclusion: Despite the advantages of probiotics as oral vaccines, their uses still include problems such as inadequate targeted colonization, diminished immune response in populations with low hygiene standards, reliance on individual microbiota, poor stability, limited efficacy, and absence of targeted immunogenicity. To address these problems, probiotics can be engineered using gene editing technologies, particularly CRISPR/Cas system. Concerns are reported regarding the safety of genetic alterations and deficiencies in efficient delivery mechanisms linked to the use of modified probiotics as oral vaccines. Further studies are needed to assess problems associated with accurate genetic alteration and efficient delivery methods to achieve the ultimate goal of further effective and safer vaccinations.

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1. Introduction

The term “probiotics” was generally specified to describe live microorganisms and substances that assist and promote intestinal microbial balances. However, this term was changed by the joint FAO/WHO group as “living microorganisms that when used in adequate contexts, provide a health benefit”. The relationship between the microbiome and human health provides new opportunities for developing novel biotherapeutics. Probiotic research has revealed a myriad of potential health benefits,

particularly in the modulation of the immune system and production of bioactive compounds for a broad spectrum of uses such as skin health, oral health and treatment of specific health conditions, including metabolic disorders, allergies, inflammatory bowel disease (IBD) and cancers. Nobel Prize was awarded to Dr. Metchnikoff at the beginning of the last century for the positive effects of probiotics and live microorganisms such as *Lactobacillus* spp. in fermented milks on human health and life span [1].

Vaccination is addressed as one of the most significant achievements in global public health. The ongoing evolution in vaccine development has introduced seven types of vaccines, including inactivated, live-attenuated, biosynthetic, toxoid, recombinant, DNA, and edible vaccines. Each type includes its own set of potential challenges, particularly regarding production complexities and high costs associated with downstream processing and purification [2]. Nowadays, probiotics are a promising generation of microorganisms used to produce edible vaccines. Recent advancements have highlighted the promise of edible vaccines as safe agents in possible pathogenicity, good mucosal and systematic immunity stimulations, and reasonable costs due to the lack of complex purification processes [3, 4]. Although probiotic vaccines are not still approved, ongoing research into the complex interplay in key agents of probiotics' immune system, microbiota, and antigens is investigated [5]. Precise molecular mechanisms of probiotic action are still under investigation. However, probiotics can affect compositions and types of bacteria in the gut due to direct affection of bacterial colonization. In recent years, studies have been carried out on the positive and negative effects of intestinal microbiota changes on development of autoimmune, autoinflammatory and infectious diseases. Furthermore, probiotics can direct the immune system to specific immune responses and specific functions to decrease inflammation in the target strains, showing their roles as vaccines based on their characteristics [6].

Recent advances in novel technology such as genetic sequencing, biomolecular biology, microbiology, bioinformatics, and medical immunology have discovered complex relationships between the microbiota of various organs and conditions of health or disease. These approaches have helped researchers look for ways to intervene in the colonization pattern of microbiota and make effective edible probiotic vaccines to improve various pathological conditions. One of the most important types of probiotic vaccines is recombinant vaccines. Genome engineering and gene editing technologies such as zinc finger nucleases (ZFN), transcription activator-like effector nucleases (TALEN), and CRISPR/Cas-linked nucleases as a bioactive field have made valuable progress in various aspects [7]. Therefore, edible probiotic vaccines with improved beneficial attributes have been engineered more frequently in recent years. Hence, the aim of this study was to introduce mucosal and systemic immune modulating potentials of probiotics as oral vaccines and use advanced genome-editing technologies to promote these microorganisms as antigen carriers and producers. Furthermore, problems in converting these microorganisms into therapeutic substitutes for current vaccinations are discussed. This study focused on the immune-modulating

role of probiotics and their interactions to increase systemic protective immunity. Furthermore, assessing the effectiveness of the engineered probiotics based on novel genome editing tools and their challenges in generation of edible probiotic vaccines were addressed.

2. Edible vaccines

Vaccination is the process of preparing the body to combat pathogens. This therapeutic approach is different from the conventional techniques of treatment, often administered post emergence of a certain disease. Vaccines not only prime the immune system to combat specific pathogens, but also provide long-lasting protection against them. Due to this function, vaccines have effectively contributed to a significant decrease in devastating infectious diseases over time [8]. Vaccines often consist of epitope/antigen structures that have restricted activity, particularly designed to activate immune system of the host without causing damages. Vaccines are often administered by injection [intramuscularly, subcutaneously, or intradermally]. However, there are limitations associated with injectable vaccines. Injectable vaccination needs disposable equipment [e.g. syringes and gloves], strict rules for transportation [e.g. cold chain] and high costs of products linked to injectable vaccines [9]. Furthermore, administration of injectable vaccinations may lead to complexities such as allergic immunological reactions and localized edema, which can eventually prevent patients from accepting the vaccine. Therefore, the use of alternative formulations has always been an important concern of scientists. With the progress in vaccine technology, the edible vaccine model emerged as an effective solution. In this type of vaccine, antigen production is carried out in various organisms such as plants, algae, silkworms, yeasts, and microorganisms known as probiotics [10]. Edible vaccines exert their immunity by immunizing the intestinal mucosa. Furthermore, edible vaccinations are appropriate for every age range and user-friendly, as they do not necessitate professionals to provide the doses [11].

3. Gut Microbiota: Functions & Benefits

Probiotics are prokaryotic microorganisms generally recognized as safe (GRAS). Probiotics possess a distinctive capacity to activate the immune system [12]. Due to their ability to express antigens and deliver them to specific immunological targets, these microorganisms include the potential as highly effective agents for vaccination. *Bacillus subtilis*, *Saccharomyces cerevisiae*, and lactic acid bacteria [LAB] are examples of probiotics commonly used in this sector [10]. In addition, introduction of interested genes has enabled the expression of foreign proteins in probiotic systems. Furthermore,

presence of antigens within cells, outside or on the surface of cells, may carefully be addressed when developing probiotics as targeted agents for immunization purposes [13]. Up to date, numerous antigens have been produced in diverse probiotic strains. Therefore, scientists have carried out extensive research to develop probiotics as targeted mucosal vaccines, demonstrating their substantial potentials for immunization. Moreover, several probiotics have clinically been assessed [14]. This development increases optimism for the shift from conventional vaccine prototypes to edible ones.

3.1 Advantages of probiotics as edible vaccines

Probiotic microorganisms possess qualities that make them promising candidates for edible vaccination treatments. Probiotics are prevalent non-colonizing microorganisms detected in the gastrointestinal tract (GIT). Thus, they include low adverse effects. Probiotic-based therapeutic vaccines offer further cost-effective alternatives to other types of immunization. The optimal dose of vaccination may readily be assessed based on the quantity of probiotics. Additionally, probiotics can include a large effect by outperforming pathogens, generating materials that are toxic to pathogens, or inhibiting their ability to attach to intestinal epithelia [15]. These microorganisms serve a dual function by producing appropriate antigens and acting as carriers for oral administration, allowing desired antigens to present to immune cells in the gut. These microorganisms are present in the bodies of individuals worldwide. Therefore, produced vaccine can be manufactured and administrated globally [10]. Due to their edible nature, this type of vaccines includes adaptations from pediatric to geriatric patients. Due to the lack of side effects associated with probiotic-based vaccines, people of any age can take probiotics at any time of the day [16]. Therefore, because of to their unique characteristics, probiotics are more appropriate candidates for edible vaccine production than others. For example, *Bacillus* spp. are distinguished by their ability to form endospores, which show exceptional resilience under hostile conditions. Hence, these attributes make them appropriate carriers for edible vaccines in dormant or vegetative forms [17]. Genetic manipulation of probiotics has been verified as reliable and effective over an extended period, demonstrating its efficacy in preventing bacterial and parasitic infections, such as malaria. Using of these engineered probiotics, which contain antigens specific to viruses, includes promises. These have been provided to fight against viral infections such as HIV, herpes virus, and influenza virus [18]. Moreover, these microorganisms may genetically be modified to enhance production of immune components, including cytokines and interleukins, which can help prevent cancers [19].

3.2 Edible probiotics and the immune response

In 1907, Elie Metchnikoff first discovered that probiotics could activate the immune system. There are several ways; by which, this interaction can be accomplished, including probiotics targeted by pattern recognition receptors (PRRs) on intestinal epithelial cells through their microorganism-associated molecular patterns. This interaction facilitated their phagocytosis, further stimulating mucosal, and systemic immunity by antigen-presenting cells (APC) activity [20]. Probiotics exert their immunogenicity by affecting intestinal immunity. Intestinal immunity is achieved by maintaining homeostasis and controlled by gut-associated lymphoid tissues, antimicrobial peptides, Peyer's patches, secretory immunoglobulins of IgA (sIgA), T cells, and various inflammatory mediators such as cytokines, chemokines, and beneficial microorganisms. The intestinal epithelial layer contains specialized cells, including entero-endocrine cells, goblet cells, paneth cells, enterocytes, tufts and M cells, which are critical components of mucosal immunity. The M cells are antigen-delivery cells that transfer luminal particles and antigens to APC. These cells are responsible for receiving antigens and subsequent transmission of the antigens to the underlying immune cells via the mechanisms of transcytosis, phagocytosis, and shedding of macrovesicles [21].

Goblet cells are linked to the process of presenting antigens to dendritic cells [22]. Dendritic cells, functioning as APCs, include an essential role in regulating cellular and humoral immune responses. By receiving antigenic subunits, these cells present them through the major histocompatibility complexes (MHC) I and II, which further activate the T cell set and create a cellular immune response through the differentiation of cell subsets such as CD4⁺ cells, macrophages, and Foxp3⁺ T-cells. They enter mesenteric lymph nodes and induce the gut-homing process [23]. Additionally, APCs stimulate activation of B cells and subsequently promote the synthesis of IgA secretory antibodies. These antibodies play a critical role in generating humoral immune responses. When probiotics are used as carriers or expressers of a particular antigen, they can stimulate specific cellular and humoral immunities associated with the antigen [24].

3.3 Engineered probiotics in edible vaccines

Using probiotics in edible vaccinations can effectively address specifically targeted immune responses that are not in conventional delivery techniques such as attenuated pathogens [25]. The edible vaccination uses probiotics as antigen carriers and adjuvants. It is important to address engineering of the immune responses to a single heterologous antigen rather than the whole microorganism [26]. The LAB strains are ideal for expressing one or more foreign proteins, particularly on their surfaces. This is

because LAB are non-pathogenic and non-invasive and can stimulate a specific immune response. Probiotics may present antigens to specific immune cells as either proteins or DNA. Hence, the probiotic bacteria transfers antigen-linked DNA into the APCs, enabling these cells to produce the active antigen. To improve efficiency, desired probiotics may genetically be modified to possess the essential mechanisms for invasion [27]. Creating and programming induction of probiotics used as edible vaccines are possible.

In response to quorum sensing signals sent by tumors, bacteria and viruses, these cells secrete specific molecules that counteract these agents. In addition, researchers are investigating and developing probiotics that specifically target the immune responses of oral mucosal pathways, including buccal, gingival, and sublingual mucosa, to enhance mucosal and systemic immune responses. Sublingual administration bypasses mucosal barriers of the GIT and may be considered as an alternative method to improve the efficacy of sensitive immunizations [28].

3.4 Highlights regarding formulation of probiotics as edible vaccines

Purified antigens are often sensitive to various mucosal barriers, resulting in the attenuation of their immunogenicity. Using probiotic bacteria as antigen producers and carriers can solve this problem in several ways, including safeguarding the target antigen in high acidic and basic environments and enhancing delivery of the target antigen to specific immune cells (M-cells in gut-associated lymphoid tissues) [29]. Edible probiotic vaccines should be assessed from several aspects, including A) stability of the formulation, since vaccine formulation is prepared orally, the most important expected goal is to stabilize formulations containing probiotics and antigens against the harsh conditions of the digestive system such as acidic and basic pH values, bile salts and enzymatic breakdowns. Thus, it is necessary to develop an edible formulation that can resist these circumstances [30]. Furthermore, it is critical to maintain the viability of the live microorganism, especially when the production of new antigens is associated with the proliferation of these germs in the host and preserving the immunogenicity of the antigen is provided through the production of an edible vaccine using probiotics. This includes precautions during processes such as lyophilization [31]. If necessary, cellular modifications should be used to enhance the microbial resistance in the relevant area. Hence, development of an optimal vaccine using probiotics needs the establishment of immunological tolerance against these microorganisms. Use of probiotics as enhancers and transport systems for delivering and administering purified antigens has grown due to technical

advancements such as MimoPath systems. An example of this scenario includes the Gram-positive *Lactococcus lactis*, which tolerates treatments under highly acidic conditions. Then, its biological components are eliminated, leading to the emergence of bacteria-like particles (BLPs). These BLPs are then combined with a pure antigen in the following stage, resulting in production of an Ag-BLP vaccine [29, 32]. B) appropriate design of the target antigen to bind to the specific immune cells and the optimal edible vaccination should have mucosal adhesion capabilities. The antigen in the vaccine must attach to a certain cell matrix such as M cells to initiate an immune response. Hence, it is critical to create edible vaccines that show mucosal adhesion qualities and effectively transport target antigens to prompt a potent immune response; and C) immune response stability, due to the limited colonization capacity of probiotics in the GIT, antigens in the edible probiotic vaccines include a short duration to induce the immune cells in the target. One solution is to co-administer an adjuvant with an edible probiotic vaccination. This can enhance the immune system activity and improve its effectiveness at the suggested site [26].

4. Genetic engineering in probiotics

Recent advancements in synthetic biology, genetic engineering, and genome sequencing technologies have strengthened the current understanding of microbial functions and promoted the development of so-called designer probiotics [33]. Investigating genome-engineering techniques has reached an innovative milestone and broadening its potential use within the medical, agricultural, and food sectors. This technology can be used for humans, provided precautions are highlighted to eradicate antibiotic resistance and ensure that modifications involve the organism's own DNA or DNA from GRAS organisms [34]. Significant uses of probiotics necessitate comprehensive genome analysis of these microorganisms. Advanced omics methodologies and system biology approaches enable researchers to optimize metabolic processes within the gut microbiome, revealing previously uncharacterized biosynthetic pathways. Such insights promote novel metabolic engineering strategies, facilitating genetic manipulation of the probiotics to address their roles in intestinal microbiota and enhance their beneficial characteristics [35]. Gene editing techniques such as site-specific recombination and the advent of innovative gene editing tools such as TALEN, ZFNs, and CRISPR/Cas9 help precise targeting and modification of specific genes (Figure 1). Merging CRISPR technology, synthetic biology and high-throughput genomic analysis represents a significant advancement in development of personalized probiotic treatments. Developing further

robust and effective CRISPR editing systems for probiotics presents two key objectives: improving probiotic traits and engineering probiotics for therapeutic uses. The first goal focuses on enhancing the inherent qualities of probiotics. This includes targeted localization of probiotics within specific niches of the gut microbiome, stress tolerance, decreased allergenicity, and virulence factors in probiotics to ensure their safety for widespread uses. The second goal emphasizes the potential of engineered probiotics for specific therapeutic functions such as autoimmune diseases, cancers, metabolic disorders, and infectious diseases. By focusing on the improvement of probiotic traits and developing targeted therapies, scientists can create safer and more effective probiotics that not only enhance individual well-being but also contribute to further understanding of microbiome interactions within the host (Figure 2) [36].

4.1 Engineered probiotic strains: therapeutic, prophylactic, and diagnostic vaccines

The field of probiotic engineering is significantly transformed, using bacterial strains that are specifically designed to colonize the GIT and produce specific molecules to serve as therapeutic, prophylactic, and diagnostic vaccines [37]. Key mechanisms of action include immunomodulation, where probiotics produce cytokines, antigens and allergens, and pathogen exclusion through the synthesis of antimicrobial peptides, biosensing capabilities useful for disease diagnosis, and metabolic modifications within the host [38]. The rational engineering of probiotics involves a systematic process that encompasses three essential steps of the selection of novel health-promoting strains, exploration of their molecular interactions with the host and its microbiota and the genetic modification to enhance or design functional characteristics that support their probiotic characteristics [39]. Several species have been investigated for their potential as vaccine vectors, effectively delivering a wide range of bacterial and viral antigens [40]. Various delivery systems, including cell wall, extracellular and cytoplasmic media, have been designed to target heterologous proteins to specific cellular locations [41]. Production of genetically modified probiotics over the past decade has expanded the scope of mucosal delivery systems to include not only prophylactic vaccines but also therapeutic agents such as enzymes, cytokines, and allergens [42]. Probiotic-based bacterial ghost vaccination represents a promising advancement in immunotherapy, particularly for DNA and protein antigen vaccines [43]. Traditional naked DNA vaccines often suffer from weak immunogenicity, which necessitates incorporation of adjuvants. However, limitations of the current adjuvants and restricted options available for recombinant vaccine manufacturers highlight necessity of alternative strategies to improve vaccine

efficacy [44]. Bacterial ghosts, which are non-viable bacterial cells that include their structural integrity, offer a unique platform for vaccine delivery. They can efficiently be internalized by various cell types, including Caco-2 cells, human dendritic cells, dendritic cells, and RAW 264.7 macrophages [45].

In a diverse group of microorganisms, *Lactobacillus* spp. are highlighted, particularly for their members, which comprise a significant portion of the human gut microbiota. Key probiotic strains, including *L. acidophilus*, *L. plantarum*, *L. johnsonii*, *L. gasseri*, *L. casei*, *L. reuteri*, *L. salivarius*, and *L. brevis*, are important for their contributions to gastrointestinal health [35]. Engineering specific *Lactobacillus* spp. to express anti-*Listeria monocytogenes* antigens [46], *L. lactis* secrete IL-10, *L. lactis* CHW9 has been used to produce the peanut Ara 2 allergen, and *L. lactis* NZ9800 has been generated to deliver the major birch allergen of Bet-v1 [47]. Furthermore, innovative methodologies have emerged in the realm of plant-probiotic interplay, wherein modified plant viruses such as tobacco mosaic virus and geminiviruses function as advanced plant expression systems and serve as vectors for delivering genetic components into plant cells [48]. In various platforms for creating edible vaccines, genetically modified microalgae, particularly *Chlamydomonas reinhardtii*, have emerged as significant candidates due to their unique characteristics and advantages, compared to traditional plant systems [49]. Developmental process of an edible vaccine using microalgae involves the insertion of a pathogen's gene of interest (GOI) into the microalgal genome through various genetic engineering techniques. Industrial benefits of microalgae such as scalability and ease of cultivation make them as further versatile options for vaccine production [50]. Gregory *et al.* have demonstrated that *C. reinhardtii* can successfully express unmodified versions of these proteins, yielding antibodies that inhibit the sexual development of malaria parasites [51]. Several engineered probiotics have reached preclinical and clinical trial phases, highlighting their potentials as edible vaccines. Noteworthy examples include recombinant *Bifidobacterium longum*, the oral bacTRL-Spike vaccine designed to immunize against coronavirus disease 2019 [52], recombinant *L. lactis* [LL-Thyy12] to treat patients with Crohn's disease [53], *L. lactis* AG019 developed for type-1 diabetes treatment [54], recombinant *L. casei* expressing the HPV16 E7 protein [55], and recombinant *L. lactis*-derived anti-malarial vaccine [56]. Clinical trials assessing the efficacy of recombinant LAB have yielded promising results, highlighting their viability as live biotherapeutics. With the current research and development, the integration of LAB in DNA vaccine delivery systems includes the

potency to revolutionize preventive and therapeutic medicines [57].

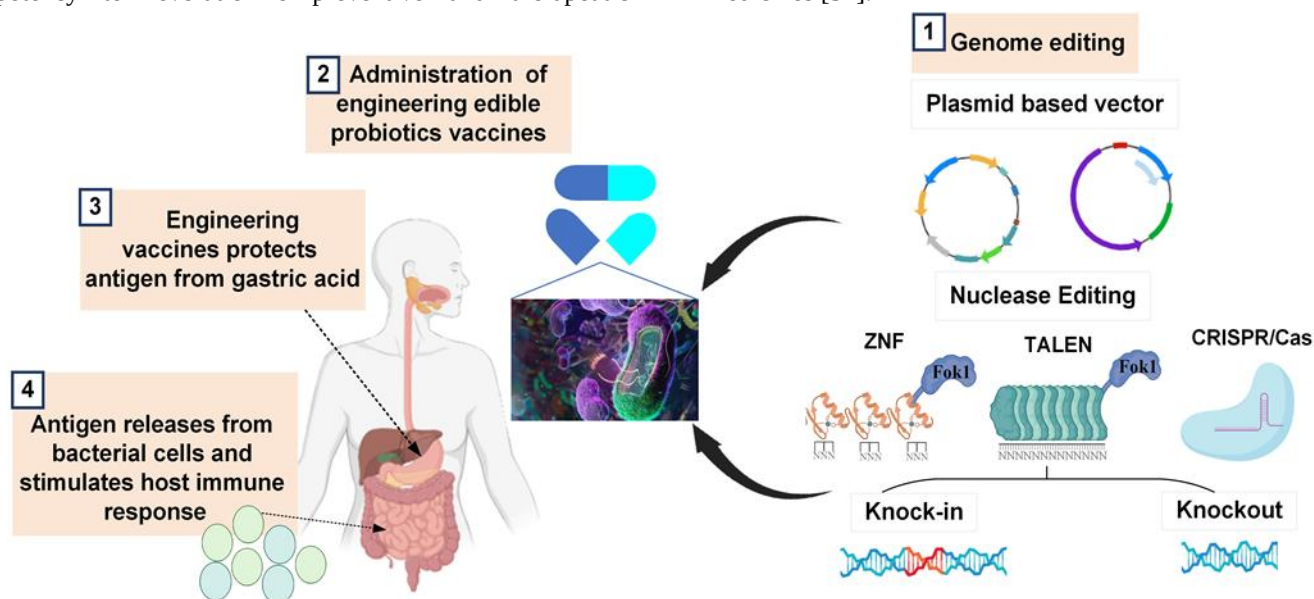


Figure 1. Engineering of edible probiotic vaccines. The figure is created using BiorRender [<https://biorender.com>]

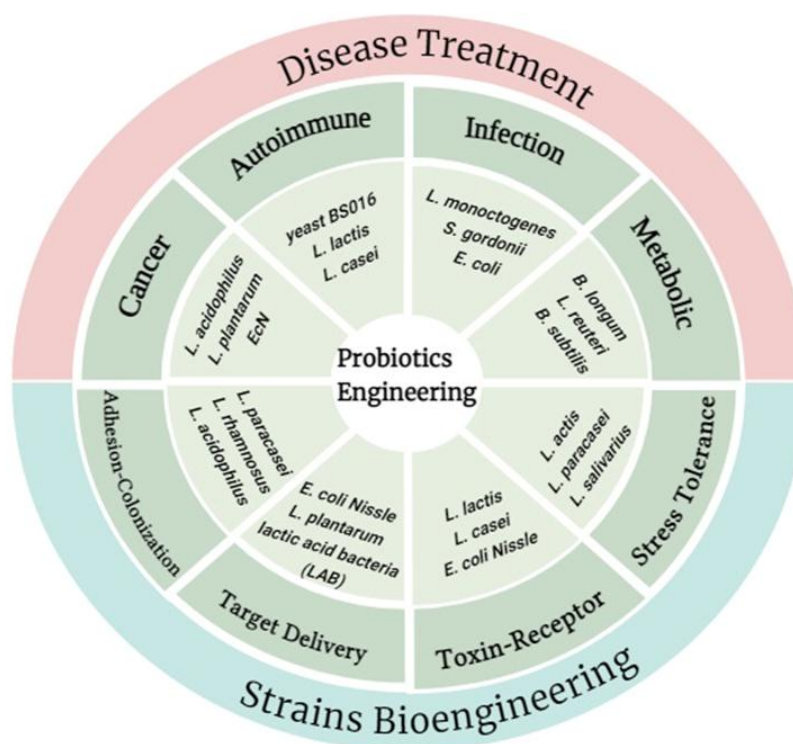


Figure 2. Use of engineered probiotic strains in disease treatment and antimicrobial resistance.

4.2 Genetic design tools used to engineer probiotics

Genome editing techniques frequently used in the engineering of probiotics include gene knockouts, gene insertions, and targeted mutations. Efficacy and precision

of various genome-editing methods play critical roles in shaping functionality and use of engineered probiotics. Probiotic engineering can be approached through two principal strategies top-down and bottom-up designs. Top-down approaches involve genome decreases, where

existing probiotic strains are simplified to create a minimal genome chassis containing essential genes for survival and metabolic functionality. In contrast, bottom-up approaches include synthesis of a minimal genome with synthetic oligonucleotides, needing careful assembly of genetic elements, metabolic pathways, and cellular membranes. Being innovative, these methods are complex and resource intensive [58].

Synthetic biology tools enhance efficiency of genomic modifications and development of counter-selectable markers has been carried out. Traditional strategies using double-stranded (dsDNA) or single-stranded (ssDNA) recombineering enable knock-in of GOI; however, limitations are reported regarding size and copy number of the inserted DNA fragments. Role of synthetic biology tools such as site-specific genome editing systems, homologous recombination, and recombineering, is critical in advancing probiotic strains. Recombineering represents a phage-derived approach that facilitates gene modification through *in vivo* homologous recombination [47]. This Red/RecET system has effectively been used in multiple *Lactobacillus* strains, allowing for fine-tuning of genomes through subtle modifications such as point mutations. In addition to these precise methodologies, strategies using non-homology based approaches such as transposon-mediated random DNA insertion broaden the scope of genetic engineering in non-model organisms. To avoid these restrictions and facilitate the integration of larger DNA constructs or clusters, site-specific recombination systems have emerged as valuable alternatives [59]. Induction of double-strand breaks (DSBs) through targeted nucleases such as ZFNs, TALEN, and RNA-guided nucleases can prompt repair mechanisms but includes risks as probiotics may show pronounced susceptibility to DSBs, leading to cell death [60].

Various methods are described to deliver genetic elements into probiotics. Direct gene delivery or vector-independent nature methods play essential roles in plant biotechnology such as biolistic, enabling the introduction of desired DNA, or RNA directly into the nuclear and chloroplast of plant cells. Chloroplast transformation, as a preferred method for producing edible vaccines, has attracted intentions owing to its ability to generate high levels of protein expression while minimizing risks of gene dispersal and horizontal gene transfer to other organisms [61]. Biolistic methods have successfully been used to develop vaccines against numerous pathogens, including cholera, Lyme disease, rotavirus, and canine parvovirus [62]. In contrast, indirect gene delivery methods use vector-mediated approaches to achieve gene transfer. A prominent example includes *Agrobacterium*-mediated gene transfer of foreign genes into the plant nucleus. This method has been set to create vaccines against various

diseases, including diarrhea, tuberculosis, dengue fever, avian flu, and Ebola [63]. Despite the promising potential of editing techniques, use of genome editing for probiotic LAB is limited, compared to model organisms such as *Escherichia coli* and *S. cerevisiae*. This limitation largely originates from stringent regulatory frameworks and cautious market response to genetically modified organisms (GMOs) [64]. Introduction of counter-selectable markers, including *upp*, *oroP*, *pheS*, and *mazF*, has revolutionized genome editing methodologies [65].

4.3 Design of gene constructs for probiotic vaccines

Construction of recombinant proteins in probiotics needs meticulous design of various components, multiple cloning sites (MCS), promoters, and terminators. Heterologous expression systems used in engineered probiotics can be categorized into two major types of constitutive and inducible. Expression systems for heterologous proteins in engineered probiotics can vary significantly, using intracellular (e.g. P_{cyt}), secretion (e.g. pSEC), or cell wall anchoring (e.g. pCWA) mechanisms [66]. Common constitutive promoters derived from the *L. lactis* genomic library include P₂₁, P₂₃, and P₅₉, which are characterized as strong promoters as well as P₃₂ and P₄₄ [67]. To lessen the overproduction of protein risk, there is a growing need for novel controlled-expression systems that act in response to external environmental factors such as temperature, bile salt concentration, pH, and antimicrobial peptides [68]. One of the most prevalent inducible systems used in probiotic engineering is the nisin-controlled gene expression (NICE) system, including key components of NisK, NisR, and NisA promoter (P_{nisA}) [69]. In addition to the NICE system, other expression systems have been developed such as the pSIP system that controls quorum sensing mechanisms, using a series of pSIP-based vectors, and promoters of class II bacteriocins such as sakacin A and sakacin P [70]. Additionally, innovative strategies such as zinc-inducible P_{znZitR} system, heat shock protein promoter-driven SICE, and auto-inducible P₁₇₀ promoter from *L. lactis* have emerged for protein expression under specific conditions [66, 71, 72]. The available research demonstrates successful uses of suicide plasmids such as the pSA3-based vector pTRK327 and elements such as IS1223 for the targeted manipulation of genomes in various probiotic LAB. These proteins often use the Sec pathway for efficient secretion, particularly when proteins possess an N-terminal signal peptide. Furthermore, protein folding and secretion facilitators such as bacterial *ffh* genes are critical in ensuring appropriate protein maturation [73]. Engineering of inducible pathways in probiotics can enable them to sense and respond to specific signals from tumors or infected cells, prompting release of therapeutic factors.

4.4 The CRISPR/Cas technology for engineering probiotic vaccines

Genetic engineering, particularly in the realm of probiotics with the potential for edible vaccination [74]. Using CRISPR, scientists can modify probiotic strains to express desired antigens, optimize metabolic pathways, and improve stability of these microorganisms under various environmental conditions. This capacity for targeted genomic intervention not only accelerates strain development but also increases potential for creating vaccines that are effective and adaptable to evolving pathogens. Moreover, use of CRISPR technology can lead to the investigation of novel probiotic candidates that were previously unreachable through conventional methods. This innovation is particularly critical in an era characterized by the rapid emergence of infectious diseases, where traditional vaccine development strategies may fail for speed and specificity. Integration of genome editing tools enhances ability to carry out extensive host-pathogen interaction studies. By creating genetically modified probiotics, researchers can better understand mechanisms underlying immune responses, creating vaccines that not only target specific pathogens but also promote overall gut health. The potential to personalize probiotics based on individual genomic profiles represents another advantage that genome editing technologies can help researchers achieve [75]. Established efficiency and specificity of CRISPR-mediated editing tools facilitate precise gene insertion, deletion, and mutation across species such as *E. coli*, *B. subtilis*, and *Lactobacillus* spp., underscoring their versatility [76]. The CRISPR/Cas systems are present in approximately 50% of the bacterial populations, including a significant prevalence in *Lactobacilli* spp. and *Bifidobacteria* spp., investigation of these systems for probiotic engineering is well-supported by natural diversity [77]. The use of exogenous CRISPR/Cas systems through plasmid-based approaches has revolutionized the capability for genetic modification in probiotic strains, particularly in those lacking inherent CRISPR/Cas systems or showing limited efficacy [78].

Recent advancements suggest that the potential of CRISPR-based editing tools in LAB is far from that fully realized, particularly with the introduction of multiple-locus editing and Cas9 non-homologous end joining (NHEJ) repair mechanisms that have successfully been implemented in other bacteria such as *E. coli* and *Mycobacterium tuberculosis* [79]. One promising achievement of research involves use of CRISPR-associated transposases (CAST) to integrate gene construct directly into chromosomal target sites without the need of positive selection, achieving integration frequencies of up to 80%. Inspired by these developments, harnessing endogenous CRISPR/Cas systems in LAB strains provides

a robust framework for genetic manipulation [80]. For example, the subtype I-E CRISPR/Cas system present in *L. crispatus* has facilitated flexible efficient genetic engineering, while specific subtypes in other LAB species such as *Pediococcus acidilactici* and *L. gasseri* have been used to enhance lactic acid production and carry out promoter replacements [81]. Recent advancements show successful implementation of CRISPR/Cas assisted genome editing in various *Lactobacillus* spp., including *L. reuteri* ATCC PTA 6475 and *L. plantarum*. Significantly, pLCNICK plasmid, developed by Song *et al.*, uses the CRISPR/Cas9D10A system for rapid genome editing in *L. casei*, demonstrating broad host compatibility for other *Lactobacillus* strains [82]. Engineered *L. plantarum* WCFS1 for N-acetylglucosamine production and enhanced galactose fermentation capabilities of *Brucella* ATCC MYA-796, illustrate the potential for engineered probiotics to provide novel health benefits [83]. Integration of RecE/T improves homologous recombination; thereby, enhancing effectiveness of CRISPR/Cas9 technologies in strain engineering [60]. Additionally, engineered CRISPR/nCas9 systems have shown successful adaptation in various *Lactobacillus* spp., indicating the broad applicability of these technologies [84]. In other probiotic strains such as *Streptococcus* spp. and *Clostridium* spp., CRISPR systems have been used to modify native traits and enhance production capabilities by executing targeted genomic edits [85]. Use of CRISPR technology is expanding beyond *Lactobacillus*, with a growing number of studies focusing on various microorganisms, including *Corynebacterium glutamicum*, *Candida parapsilosis*, and *E. coli* [76]. Versatility of Cas9 nickase (nCas9) and dead Cas9 (dCas9) tools enables precise genome editing without inducing double-strand breaks, while Cas13 nucleases provide a unique mechanism for RNA cleavage, further expanding the toolkit available for microbial genetic engineering. One of the pioneering companies, Zbio, has emerged as the first to commercialize genetically engineered probiotic products. In addition to LAB, CRISPR/Cas systems have verified advantageous for yeasts such as *S. cerevisiae* and *S. boulardii* [86].

4.5 Defense mechanisms of bacteria against foreign DNA: implications for genome editing

Bacteria have developed sophisticated defense mechanisms to protect their genomic integrity from the invasion of foreign DNA. Two primary systems that exemplify this defensive strategy include restriction-modification systems and CRISPR/Cas systems. Over 90% of bacteria possess defense mechanisms such as restriction-modification systems, underscoring their importance in safeguarding microbial genomes against exogenous genetic materials. The CRISPR/Cas systems, detected in

approximately 85.2% of archaea and 42.3% of bacteria, present another layer of complexity in the landscape of microbial defense against invasive DNAs [87]. Within the *Lactobacillus* genus that includes a significant representation of probiotics, occurrence of CRISPR/Cas systems is significantly high. The significance of these defense mechanisms becomes particularly evident in the context of genome editing techniques, which necessitate the successful integration of foreign DNA into bacterial genomes. To evade bacterial defenses, researchers have suggested three distinct strategies. First, using an intermediate host that boasts compatible methylation patterns, facilitating transformation processes. Second, using recombinant intermediate hosts that express the requisite methyltransferases anticipated in the target microorganism [57]. Third, another innovative approach involves *in vitro* incubation of foreign DNA with commercial methyltransferases to match the host's DNA methylation patterns, a technique that has significantly improved transformation efficiency in species such as *L. plantarum*. By effectively overcoming these bacterial defense mechanisms, researchers can enhance efficiency and reliability of the genetic modifications in bacteria.

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5. Improvement of Probiotic Efficacy Through Bioengineering

5.1. Enhancement of colonization exclusion

Increasing occurrence of pathogenic bacteria and their subsequent infections necessitates innovative strategies for prevention and management. One promising approach is the enhanced colonization of probiotics in the intestinal mucosal surface, which can create a competitive environment that limits colonization of harmful microorganisms. Genetic modification of adhesions, flagellins and fimbriae, help probiotics to serve as effective barriers to pathogen colonization in the GIT [89]. For example, the internalin A gene from *L. monocytogenes* expressed in the probiotic *L. lactis*, a recombinant *L. paracasei* strain that expressed the *Listeria* adhesion protein (LAP) suppresses the adhesion of *L. monocytogenes* [89]. Expressing the surface-associated flagellin of *B. cereus* in *L. lactis*, to competitive inhibition of pathogenic bacteria such as *E. coli* and *Salmonella enterica* from binding to intestinal epithelia [90].

5.2. A focus on stress tolerance

Numerous probiotic strains show limited tolerance to environmental stresses such as temperature, salinity and oxygen level. These limitations lessen stability and effectiveness of the probiotics as edible vaccines. Through genetic modification, researchers aim to enhance stress adaptation capabilities of the probiotics; thus, ensuring their viability and therapeutic efficacy [38]. A major key to this approach includes manipulation of heat-shock proteins, particularly GroES and GroEL, which are critical for the probiotic viability within various temperatures. A study by Desmond *et al.* demonstrated that overexpressing these chaperones in *L. paracasei* NFBC338 significantly improved its thermotolerance and solvent resistance, suggesting that strategic gene modifications can enhance robustness of the probiotic strains. Moreover, investigation of heterologous gene expression further contributes to probiotic resilience. For example, expression of the *BetL* gene in *L. salivarius* UCC118 through a nisin-controlled system demonstrated enhanced resistance to various environmental stresses [91]. Similarly, research involving *Bifidobacterium breve* UCC2003, which harbored the *BetL* gene, revealed an enhanced tolerance to gastric acidity and high osmolarity, indicating that genetic adaptations could significantly improve stress tolerance [92]. Additionally, cloning of the trehalose synthesis gene (*ostAB*) from *E. coli* into *L. lactis* has demonstrated improved survival rates for probiotics during freeze-drying processes and in high bile concentrations. These findings verify feasibility of using genes from pathogenic organisms to enhance stress resilience of the beneficial probiotics.

5.3. Receptor mimicry probiotic system

Understanding of the precise dynamics of toxin-receptor interactions on the surface of human intestinal cells provides a foundation for developing innovative strategies aimed to interrupting pathogen adhesion and disrupting pathogen; thereby, decreasing incidence and severity of infections [93]. One promising therapeutic approach involves expression of toxin receptors on the surface of probiotic strains to mimic the host's receptor environment. Such receptor mimics can produce lipopolysaccharides recognized by toxins such as cholera toxin or enterotoxigenic *E. coli* (ETEC) heat-labile toxin. Expression of *Neisseria gonorrhoeae* galactosyltransferase genes in non-pathogenic *E. coli* strains resulted in a 100% efficacy rate in treating lethal infections caused by Shigatoxigenic *E. coli* (STEC) in mice [94]. Similarly, expression of a glycosyltransferase gene specific to meningococcal toxins in probiotic *E. coli* created a competitive environment that inhibited pathogen binding and hence subsequent infections [93]. Mimicking of host receptors on probiotic surfaces can serve as an effective strategy for preventing specific toxin-mediated pathologies

[95]. Moreover, concept of disrupting pathogen virulence through innovative means such as quorum sensing manipulation has attracted intentions. As seen for *E. coli* Nissle 1917 strain producing cholera autoinducer 1 (CAI-1), interference with virulence gene expression upon recognition of specific signaling molecules decreased colonization of *Vibrio cholerae* in experimental models [96].

5.4. The promising landscape of next-generation probiotics

The objective is to develop next-generation probiotics (NGPs) that offer enhanced therapeutic potentials, compared to those traditional probiotics. The integration of computational biology with genetic engineering is particularly advantageous, as it supports the design of next-generation synthetic LAB capable of overcoming challenges associated with industrial scale-up and complex biologics production. A relevant attempt in this field includes the Lactochassis project, which aims to create synthetic LAB, specifically for biomedical uses. This initiative underscores a paradigm shift in probiotic development, transitioning conventional probiotics from mere vectors for therapeutic delivery to engineered microbial ‘physicians’ that actively respond to health challenges [58]. Certain strains show significant promises due to their effects on health and disease. Good examples are *Eubacterium hallii*, *Faecalibacterium prausnitzii*, *Roseburia* spp., *Akkermansia muciniphila*, and *Bacteroides fragilis*. Research suggests that these microbes play vital roles in key physiological processes, including modulation of gastrointestinal immunity, enhancement of immunotherapy efficacy in cancer patients, maintenance of intestinal barrier integrity, and generation of beneficial metabolites, particularly short-chain fatty acids (SCFAs). Despite their promising potentials, widespread use of these NGPs produces significant problems. Key challenges include development of appropriate methods for culturing and storing these oxygen-sensitive organisms, which complicates their integration into consumer products [97]. As microbiome research evolves, efforts are made to genetically engineer or modify these NGPs, enabling them to target specific diseases or health conditions more effectively. Uses of this technology range from edible health to the management of allergies, metabolic disorders, cancers, and IBD. Engineered probiotics for edible vaccines in the treatment of diseases are represented in Table 1.

6. Challenges Associated with Engineered Probiotics as Edible Vaccines

Advances in probiotic-based edible vaccine technology have reached the clinical stage [14]. However, there are still several problems in this way. Edible probiotic vaccines may decrease the immune response in populations with low hygiene and increases in the occurrence of illnesses associated with harmful microorganisms. These germs dominate the symbiotic community with other microbiota because of the weakened immune system of the human body and specific manifestation of the disease. Furthermore, variety in microbiota in individuals residing in a certain area includes a significant effect on the interactions with probiotics. Thus, assessing appropriate reactions to the edible probiotic vaccines becomes further challenging. Another critical concern includes health of the digestive system. Intestinal dysfunction can weaken the immune response. Moreover, idiopathic variables such as environmental enteropathy, a condition that damages intestinal health with no recognized origin, are reported. These factors are accountable for the diminished responses to vaccines [122].

In elderly people, cellular and humoral immune responses are weakened and these factors were accountable for decreasing vaccine protective efficacy. The next challenge includes non-colonizing nature of the probiotics, which needs daily treatment with high doses (approximately 100 times the injection dose) to have specific health benefits for the host, due to the variable pH values in various parts of the gut. Therefore, improvement of parameters linked to microorganisms and formulation should be addressed to overcome this challenge [30, 123]. In protein-based vaccinations, selecting an appropriate strain for protein expression is difficult because of the genetic variability linked to probiotic strains. Various species show distinct patterns of antigen expression, making it challenging to select the optimal pattern. One further concern includes the absence of post-translational processes in prokaryotes. For example, when the target antigen is overexpressed in the probiotic bacterial species *E.coli*, it leads to the formation of inclusion bodies (IBs). To achieve optimal effectiveness, these IBs need further processing to unfold and refold desired proteins to back to their original conditions. This is a separate process and needs extra financial resources. In addition, developing a reliable protocol for modifying proteins after their expression during production of vaccines is inherently difficult. This is because various post-translational mechanisms may be necessary depending on the desired structures and functional behaviors of the protein antigens [10]

Table1. Engineered probiotics for edible vaccines in the treatment of various diseases

Engineered probiotic	Target Genes/antigen	Disease/ Infection	Genome engineering Tools	Result	Ref.
<i>Listeria monocytogenes</i>	P18, gp160	HIV	Synthetic peptide	Induction of CTL against HIV	[98]
<i>Streptococcus gordonii</i>	gp120 /V3 domain	HIV	Plasmids-based	V3-specific IgG2a antibodies release	[99]
<i>L. casei</i>	PA	Anthrax	Plasmids-based	Induction humoral and cellular immune responses	[100]
<i>L. lactis</i>	PppA [LPA+]	Streptococcus pneumoniae	Plasmids-based	Boosted resistance to intranasal infection	[101]
<i>EcN</i>	Stx B-subunits, OspA and OspG proteins	ETEC	Plasmids-based	Insufficient to mediate protection	[102]
<i>Streptococcus gordonii</i>	S1 subunit of pertussis toxin [PT]	Bordetella pertussis	Plasmids-based	Elicited mucosal antibodies against PT and SpaP	[103]
<i>Lactiplantibacillus plantarum</i>	SARS-CoV-2 spike protein[S]	SARS-CoV-2	Plasmids-based	SARS-CoV-2 vaccine	[104]
<i>Lactiplantibacillus plantarum</i>	Oncofetal antigen [OFA]	Cancer vaccine	Plasmids-based	Induced a specific immune response against OFA	[105]
<i>L. lactis</i>	Glucagon-like peptide [GLP] 1 [1-37]	Pancreatic β -cell	Plasmids-based	Successful oral delivery of GLP-1	[106]
<i>L. reuteri</i>	Phenylalanine lyase	Phenylketonuria [PKU]	Plasmids-based	Potential treatment for PKU	[107]
<i>L. lactis</i>	Cathelicidin	Colitis	Plasmids-based	Elicit antigen-specific tolerance	[108]
<i>L. lactis</i>	Heat-shock protein 65 [Hsp65]	Atherosclerosis	Plasmids-based	Elicit antigen-specific tolerance Protective effect on atherosclerotic and endothelial damage	[109]
<i>E. coli</i> <i>B. subtilis</i>	Csenolase	Clonorchis sinensis	Plasmids-based	Killed spores triggered reactive Th1/Th2 immune response and exhibited protective efficacy against C. sinensis infection	[110]
<i>L. lactis</i>	GLP-1 [1–37]	Alzheimer's disease	Plasmids-based	Reduce inflammatory cytokine, glia activation&amyloid-b accumulation	[111]
<i>EcN</i>	alr and dadX genes	P. aeruginosa	phage λ Red recombinase	<i>In vivo</i> prophylactic and therapeutic activity against P. aeruginosa	[112]
<i>Yeast strain BS016</i>	P2Y2	IBD	homologous recombination	Suppressed intestinal inflammation in mouse models of IBD	[113]
<i>E. coli</i>	tet [X4] and mcr-1 genes	Antimicrobials resistance	CRISPR/Cas9	Significantly decrease the counts of tigecycline and colistin-resistant bacteria to 1% <i>in vivo</i>	[114]
<i>EcN</i>	Several genes	Antibiotic-resistant bacteria	CRISPR/Cas9	Eliminates antibiotic-resistant bacteria in the gut	[115]
<i>EcN</i>	F4 and F18 fimbriae cluster	ETEC	CRISPR/Cas9	Defend against the infection of F4+/F18+ ETEC	[116]
<i>B. subtilis</i>	SCK6	Obesity	CRISPR/Cas9	Regulating metabolic to prevent obesity	[117]
<i>EcN</i>	MPER	HIV	CRISPR/Cas9	A potential platform for vaccine antigen	[118]
<i>Saccharomyces boulardii</i>	Ovalbumin	<i>Saccharomyces boulardii</i>	CRISPR/Cas9	Promising tool for oral delivery of vaccines or therap-eutic proteins	[119]
<i>C. butyricum</i> <i>C. sporogenes</i>	NYESO1/CTAG1 Spike_S1 antigens	Cancer vaccine SARS-CoV-2	CRISPR/FnCas12a	Potential platform for cancer & anti-viral vaccine Ag	[120]
<i>L. paracasei</i>	S1/ Δ Alr HLJ-27	PEDV	CRISPR/nCas9	Induce mucosal, humoral, &cellular immune responses against PEDV	[121]
<i>EcN</i>	F4 and F18 fimbriae cluster	ETEC	CRISPR/Cas9	Defend against the infection of F4+/F18+ ETEC	[116]

Due to their inherent nature, protein-based edible probiotic vaccines must overcome physical-chemical barriers such as pH and bile salts, as well as biological barriers such as intestinal-epithelial barriers. To ensure effectiveness and safety of drug delivery, it is essential to carry out comprehensive studies on formulations to overcome difficulties associated with oral administration of medicines [124]. Plasmids are the predominant approach used in probiotic manipulation. Relatively, type of the probiotic genetic manipulation is important. Lifespan of the plasmids in probiotics is limited, leading to a gradual decrease in production of the desired protein. Hence, development of further appropriate alternatives is necessary to design probiotics and efficient desired antigen production. For plasmids, horizontal transfer of genetically modified genes and antibiotic resistance of the probiotics present significant hazards. Hence, it is critical to use conservation strategies with the progress of these GMOs. Probiotics engineering using novel genome editing tools provides a promising solution to overcome limitations and expanding their clinical uses. The CRISPR approach has been highlighted as an innovative technological tool that helps in development of novel and highly targeted edible vaccines. The quick progress and critical steps in CRISPR/Cas9 use include a wide range, converting it into a significant area of biotechnology. In addition to the significant knowledge achieved, several fundamental issues are still unresolved. The use of CRISPR technology includes substantial barriers that involve concerted efforts for resolution. It is important to address these challenges before the technology integrates into clinical settings. A forward-looking perspective urges a concerted focus on extensive research studies to thoroughly assess efficacy and safety of CRISPR-based treatment protocols, specifically for edible vaccines. Moreover, combining CRISPR with complementary technologies such as nanotechnology may further enhance effectiveness of the probiotics, enriching knowledge of edible vaccines, and opening new horizons for novel innovative solutions in immunization [125].

7. Conclusion

Findings of this study demonstrate the potential of engineered probiotics as a revolutionary solution in immunization. Using advanced genetic tools such as CRISPR/Cas genome editing and synthetic biology, these modified microorganisms can act as carriers and producers of specific antigens, providing a novel approach to vaccine delivery. Probiotic-based oral vaccines include significant advantages such as targeted antigen delivery and enhancement of mucosal and systemic immune responses. Furthermore, these vaccines are affordable for the general

public. The potential of engineering probiotics for preventive and therapeutic uses to change the prescription pattern from traditional injectable vaccines to oral alternatives makes their uses further customer-friendly and fit with modern health care needs. Despite these promising advances, several challenges are still addressed, including variations in immune responses in various individuals dependence on the health of the GIT, instability of the probiotics in various gastrointestinal environments, and possibility of the potential horizontal transfer of genetically modified genes. Addressing these challenges needs comprehensive studies to optimize probiotic formulations, including use of nanotechnology methods and ensuring their safety through advanced genetic techniques. This facilitates further sustainable, user-friendly immunization strategies globally that are fit with emerging public health demands.

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9. Author Contributions

N.H., F.SH., E.SH. and M.BV. wrote the manuscript and F.SH. and E.SH. revised the manuscript.

10. Conflict of Interest

The authors declare no conflict of interest.

References

1. Idrees M, Imran M, Atiq N, Zahra R, Abid R, Alreshidi M, Roberts T, Abdelgadir A, Tipu MK, Farid A: Probiotics, their action modality and the use of multi-omics in metamorphosis of commensal microbiota into target-based probiotics. *Front Nutr.* 2022, 9:959941.
<https://doi.org/10.3389/fnut.2022.959941>
2. Saleh A, Qamar S, Tekin A, Singh R, Kashyap R: Vaccine development throughout history. *Cureus* 2021, 13[.].
<https://doi.org/10.7759/cureus.16635>
3. Esmael H, Hirpa E: Review on edible vaccine. *Acad J Nutr.* 2015, 4[1]:40-49
<https://doi.org/10.5829/idosi.ajn.2015.4.1.956>
4. Foroutan NS, Tabandeh F, Khodabandeh M, Mojjani N, Maghsoudi A, Moradi M: Isolation and Identification of an Indigenous Probiotic Lactobacillus Strain: Its Encapsulation with Natural Branched Polysaccharids to Improve Bacterial Viability. *Appl Food Biotechnol.* 2017, 4[3]:133-1.4.
<https://doi.org/10.22037/afb.v4i3.16471>
5. Fong FLY, Shah NP, Kirjavainen P, El-Nezami H: Mechanism of action of probiotic bacteria on intestinal and systemic immunities and antigen-presenting cells. *Int Rev Immunol.* 2016, 35[3]:179-188.
<https://doi.org/10.3109/08830185.2015.1096937>

6. La Fata G, Weber P, Mohajeri MH :Probiotics and the gut immune system: indirect regulation. *Probiotics Antimicrob Proteins*. 2018, 10:11-21.
<https://doi.org/10.1007/s12602-017-9322-6>
7. Dixit S, Kumar A, Srinivasan K, Vincent PDR, Ramu Krishnan N: Advancing genome editing with artificial intelligence: opportunities, challenges and future directions. *Front Bioeng Biotechnol*. 2024, 11:1335901.
<https://doi.org/10.3389/fbioe.2023.1335901>
8. Sautto GA, Kirchenbaum GA, Diotti RA, Criscuolo E, Ferrara F: Next generation vaccines for infectious diseases. *J Immunol Res*. 2019;2019:5890962.
<https://doi.org/10.1155/2019/5890962>
9. Criscuolo E ,Caputo V, Diotti R, Sautto G, Kirchenbaum G, Clementi N: Alternative methods of vaccine delivery: an overview of edible and intradermal vaccines. *J Immunol Res*. 2019;2019:8303648.
<https://doi.org/10.1155/2019/8303648>
10. Rosales-Mendoza S, Angulo C, Meza B: Food-grade organisms as vaccine biofactories and oral delivery vehicles. *Trends Biotechnol*. 2016, 34[2]:124-136.
<https://doi.org/10.1016/j.tibtech.2015.11.007>
11. O'Hagan DT: Microparticles and polymers for the mucosal delivery of vaccines. *Adv Drug Deliv Rev*. 1998, 34[2-3]:305-320.
[https://doi.org/10.1016/s0169-409x\[98\]00045-3](https://doi.org/10.1016/s0169-409x[98]00045-3)
12. Oh J-H, van Pijkeren J-P: CRISPR–Cas9-assisted recombineering in *Lactobacillus reuteri*. *Nucleic Acids Res*. 2014, 42[17]:e131-e131.
<https://doi.org/10.1093/nar/gku623>
13. Jiang B, Li Z, Ou B, Duan Q, Zhu G: Targeting ideal oral vaccine vectors based on probiotics: a systematical view. *Appl Microbiol Biotechnol*. 2019, 103:3941-3953.
<https://doi.org/10.1007/s00253-019-09770-7>
14. LeCureux JS, Dean GA: *Lactobacillus* mucosal vaccine vectors: immune responses against bacterial and viral antigens. *Msphere* 2018, 3[3]:10.1128/msphere. 00061-00018.
<https://doi.org/10.1128/mSphere.00061-18>
15. MacDonald TT, Bell I: Probiotics and the immune response to vaccines. *Proc Nutr Soc*. 2010, 69[3]:442-446.
<https://doi.org/10.1017/S0029665110001758>
16. Alimolaei M, Golchin M, Ezatkah M: Orally administered recombinant *Lactobacillus casei* vector vaccine expressing β -toxoid of *Clostridium perfringens* that induced protective immunity responses. *Res Vet Sci*. 2017, 115:332-339.
<https://doi.org/10.1016/j.rvsc.2017.06.018>
17. Amuguni H, Tzipori S: *Bacillus subtilis*: a temperature resistant and needle free delivery system of immunogens. *Hum Vaccin Immunother*. 2012, 8[7]:979-984.
<https://doi.org/10.4161/hv.20694>
18. Al Kassaa I, AL KASSAA I: Antiviral probiotics: a new concept in medical sciences. New insights on antiviral probiotics: From research to applications. Springer, Cham. 2017:1-46.
https://doi.org/10.1007/978-3-319-49688-7_1
19. Zhang W, Azevedo MS, Wen K, Gonzalez A, Saif LJ, Li G, Yousef AE, Yuan L: Probiotic *Lactobacillus acidophilus* enhances the immunogenicity of an oral rotavirus vaccine in gnotobiotic pigs. *Vaccine*. 2008, 26[29-30]:3655-3661.
<https://doi.org/10.1016/j.vaccine.2008.04.070>
20. van Baarlen P, Wells JM, Kleerebezem M: Regulation of intestinal homeostasis and immunity with probiotic lactobacilli. *Trends Immunol*. 2013, 34[5]:208-215.
<https://doi.org/10.1016/j.it.2013.01.005>
21. Ramirez JEV, Sharpe LA, Peppas NA: Current state and challenges in developing oral vaccines. *Adv Drug Deliv Rev*. 2017, 114:116-131.
<https://doi.org/10.1016/j.addr.2017.04.008>
22. Allaire JM, Crowley SM, Law HT, Chang S-Y, Ko H-J , Vallance BA: The intestinal epithelium: central coordinator of mucosal immunity. *Trends Immunol*. 2018, 39[9]:677-696.
<https://doi.org/10.1016/j.it.2018.04.002>
23. Tokuhara D, Kurashima Y, Kamioka M, Nakayama T, Ernst P, Kiyono H: A comprehensive understanding of the gut mucosal immune system in allergic inflammation. *Allergol Int*. 2019, 68[1]:17-25.
<https://doi.org/10.1016/j.alit.2018.09.004>
24. Owen JL, Sahay B, Mohamadzadeh M: New generation of oral mucosal vaccines targeting dendritic cells. *Curr Opin Chem Biol*. 2013, 17[6]:918-924.
<https://doi.org/10.1016/j.cbpa.2013.06.013>
25. Barhate G, Gautam M, Gairola S, Jadhav S, Pokharkar V: Enhanced mucosal immune responses against tetanus toxoid using novel delivery system comprised of chitosan functionalized gold nanoparticles and botanical adjuvant: Characterization, immunogenicity and stability assessment. *J Pharm Sci* 2014, 103[11]:3448-3456.
<https://doi.org/10.1002/jps.24161>
26. Woodrow KA, Bennett KM, Lo DD: Mucosal vaccine design and delivery. *Annu Rev Biomed Eng*. 2012, 14[1]:17-46.
<https://doi.org/10.1146/annurev-bioeng-071811-150054>
27. Hu Q, Wu M, Fang C, Cheng C, Zhao M, Fang W, Chu PK , Ping Y, Tang G: Engineering nanoparticle-coated bacteria as oral DNA vaccines for cancer immunotherapy. *Nano Lett*. 2015, 15[4]:2732-2739.
<https://doi.org/10.1021/acs.nanolett.5b00570>
28. Van Overtvelt L, Moussu H, Horiot S, Samson S, Lombardi V, Mascarell L, Van de Moer A, Bourdet-Sicard R, Moingeon P: Lactic acid bacteria as adjuvants for sublingual allergy vaccines. *Vaccine*. 2010, 28[17]:2986-2992.
<https://doi.org/10.1016/j.vaccine.2010.02.009>
29. Singh M: Novel immune potentiators and delivery technologies for next generation vaccines: Springer Science & Business Media; 2013.
<https://doi.org/10.1007/978-1-4614-5380-2>
30. Govender M, Choonara YE, Kumar P, du Toit LC, van Vuuren S, Pillay V: A review of the advancements in probiotic delivery: Conventional vs. non-conventional formulations for intestinal flora supplementation. *Aaps PharmSciTech* 2014, 15:29-43.
<https://doi.org/10.1208/s12249-013-0027-1>
31. Huyghebaert N, Vermeire A, Neiryck S, Steidler L, Remaut E, Remon JP: Development of an enteric-coated formulation containing freeze-dried, viable recombinant *Lactococcus lactis* for the ileal mucosal delivery of human interleukin-10. *Eur J Pharm Biopharm*. 2005, 60[3]:349-359.
<https://doi.org/10.1016/j.ejpb.2005.02.012>
32. van Roosmalen ML, Kanninga R, El Khattabi M, Neef J, Audouy S, Bosma T, Kuipers A, Post E, Steen A, Kok J: Mucosal vaccine delivery of antigens tightly bound to an

- adjuvant particle made from food-grade bacteria .Methods 2006, 38[2]:144-149.
<https://doi.org/10.1016/j.ymeth.2005.09.015>
33. Hag S, Poondla N: Genetically engineered probiotics. Probiotic Research in Therapeutics: Volume 1: Applications in Cancers and Immunological Diseases 2021:295-328.
https://doi.org/10.1007/978-981-15-8214-1_16
34. Bravo D, Landete JM: Genetic engineering as a powerful tool to improve probiotic strains. Biotechnol Genet Eng Rev. 2017, 33[2]:173-189.
<https://doi.org/10.1080/02648725.2017.1408257>
35. Yadav R, Kumar V, Baweja M, Shukla P: Gene editing and genetic engineering approaches for advanced probiotics: a review. Crit Rev Food Sci Nutr. 2018, 58[10]:1735-1746.
<https://doi.org/10.1080/10408398.2016.1274877>
36. Gupta RM, Musunuru K: Expanding the genetic editing tool kit: ZFNs, TALENs and CRISPR-Cas9. J Clin Invest. 2014, 124[10]:4154-4161.
<https://doi.org/10.1172/JCI72992>
37. Braff D, Shis D, Collins JJ: Synthetic biology platform technologies for antimicrobial applications. Adv Drug Deliv Rev. 2016, 105:35-43.
<https://doi.org/10.1016/j.addr.2016.04.006>
38. Mathipa MG, Thantsha MS: Probiotic engineering: towards development of robust probiotic strains with enhanced functional properties and for targeted control of enteric pathogens. Gut Pathog. 2017, 9:1-17.
<https://doi.org/10.1186/s13099-017-0178-9>
39. Zuo F, Chen S, Marcotte H: Engineer probiotic bifidobacteria for food and biomedical applications-Current status and future prospective. Biotechnol Adv. 2020, 45:107654.
<https://doi.org/10.1016/j.biotechadv.2020.107654>
40. Wells J, Robinson K, Chamberlain L, Schofield K, Le Page R: Lactic acid bacteria as vaccine delivery vehicles. Antonie Van Leeuwenhoek. 1996, 70:317-330.
<https://doi.org/10.1007/BF00395939>
41. Le Loir Y, Azevedo V, Oliveira SC, Freitas DA, Miyoshi A, Bermúdez-Humarán LG, Nouaille S, Ribeiro LA, Leclercq S, Gabriel JE: Protein secretion in Lactococcus lactis: an efficient way to increase the overall heterologous protein production. Microb Cell Fact. 2005, 4:1-13.
<https://doi.org/10.1186/1475-2859-4-2>
42. Wells J: Mucosal vaccination and therapy with genetically modified lactic acid bacteria. Annu Rev Food Sci Technol. 2011, 2[1]:423-445.
<https://doi.org/10.1146/annurev-food-022510-133640>
43. Chen H, Ji H, Kong X, Lei P, Yang Q, Wu W, Jin L, Sun D: Bacterial ghosts-based vaccine and drug delivery systems. Pharmaceutics. 2021, 13[11]:1892.
<https://doi.org/10.3390/pharmaceutics13111892>
44. Xu Y, Yuen P-W, Lam JK-W :Intranasal DNA vaccine for protection against respiratory infectious diseases: the delivery perspectives. Pharmaceutics. 2014, 6[3]:378-415.
<https://doi.org/10.3390/pharmaceutics6030378>
45. Stein E, Inic-Kanada A, Belij S, Montanaro J, Bintner N, Schlacher S, Mayr UB, Lubitz W, Stojanovic M, Najdenski H: In vitro and in vivo uptake study of Escherichia coli Nissle 1917 bacterial ghosts: cell-based delivery system to target ocular surface diseases. Invest Ophthalmol Vis Sci. 2013, 54[9]:6326-6333.
<https://doi.org/10.1167/iovs.13-12044>
46. Drolia R, Amalaradjou MAR, Ryan V, Tenguria S, Liu D, Bai X, Xu L, Singh AK, Cox AD, Bernal-Crespo V: Receptor-targeted engineered probiotics mitigate lethal Listeria infection. Nat Commun. 2020, 11[1]:6344.
<https://doi.org/10.1038/s41467-020-20200-5>
47. Wu J, Xin Y, Kong J, Guo T: Genetic tools for the development of recombinant lactic acid bacteria. Microb Cell Fact. 2021, 20[1]:118.
<https://doi.org/10.1186/s12934-021-01607-1>
48. Fujiki M, Kaczmarczyk JF, Yusibov V, Rabindran S: Development of a new cucumber mosaic virus-based plant expression vector with truncated 3a movement protein. Virology. 2008, 372-379: [1]381 ,
<https://doi.org/10.1016/j.virol.2008.08.022>
49. Jiji MG, Ninan MA, Thomas V, Thomas BT: Edible microalgae: potential candidate for developing edible vaccines. Vegetos. 2023:1-6.
<https://doi.org/10.1007/s42535-023-00636-y>
50. Gangl D, Zedler JA, Rajakumar PD, Martinez EMR, Riseley A, Włodarczyk A, Purton S, Sakuragi Y, Howe CJ, Jensen PE: Biotechnological exploitation of microalgae. J Exp Bot. 2015, 66[22]:6975-6990.
<https://doi.org/10.1093/jxb/erv426>
51. Gregory JA, Li F, Tomosada LM, Cox CJ, Topol AB, Vinetz JM, Mayfield S: Algae-produced Pfs25 elicits antibodies that inhibit malaria transmission. PloS one 2012, 7[5]:e37179.
<https://doi.org/10.1371/journal.pone.0037179>
52. Silveira MM, Moreira GMSG, Mendonça M: DNA vaccines against COVID-19: Perspectives and challenges. Life Sci. 2021, 267:118919.
<https://doi.org/10.1016/j.lfs.2020.118919>
53. Braat H, Rottiers P, Hommes DW, Huyghebaert N, Remaut E, Remon JP, Van Deventer SJ, Neirynck S, Peppelenbosch MP, Steidler L: A phase I trial with transgenic bacteria expressing interleukin-10 in Crohn's disease. Clin Gastroenterol Hepatol. 2006, 4[6]:754-759.
<https://doi.org/10.1016/j.cgh.2006.03.028>
54. Mathieu C, Wiedeman A, Cerosaletti K, Long SA, Serti E, Cooney L, Vermeiren J, Caluwaerts S, Van Huynegem K, Steidler L: A first-in-human, open-label Phase 1b and a randomised, double-blind Phase 2a clinical trial in recent-onset type 1 diabetes with AG019 as monotherapy and in combination with teplizumab. Diabetologia. 2024, 67[1]:27-41.
<https://doi.org/10.1007/s00125-023-06014-2>
55. Komatsu A, Igimi S, Kawana K: Optimization of human papillomavirus [HPV] type 16 E7-expressing lactobacillus-based vaccine for induction of mucosal E7-specific IFN γ -producing cells. Vaccine. 2018, 36[24]:3423-3426.
<https://doi.org/10.1016/j.vaccine.2018.05.009>
56. Sirima SB, Mordmüller B, Milligan P, Ngoa UA, Kironde F, Atuguba F, Tiono AB, Issifou S, Kaddumukasa M, Bangre O: A phase 2b randomized, controlled trial of the efficacy of the GMZ2 malaria vaccine in African children. Vaccine. 2016, 34[38]:4536-4542.
<https://doi.org/10.1016/j.vaccine.2016.07.041>
57. Mugwanda K, Hamese S, Van Zyl WF, Prinsloo E, Du Plessis M, Dicks LM, Thimiri Govinda Raj DB: Recent advances in genetic tools for engineering probiotic lactic acid bacteria. Biosci Rep. 2023, 43[1]:BSR20211299.

<https://doi.org/10.1042/BSR20211299>

58. Romero-Luna HE, Hernández-Mendoza A, González-Córdova AF, Peredo-Lovillo A: Bioactive peptides produced by engineered probiotics and other food-grade bacteria: A review. *Food Chem X*. 2022, 13:100196.
<https://doi.org/10.1016/j.fochx.2021.100196>
59. Yang P, Wang J, Qi Q: Prophage recombinases-mediated genome engineering in *Lactobacillus plantarum*. *Microb Cell Fact*. 2015, 14:1-11.
<https://doi.org/10.1186/s12934-015-0344-z>
60. Huang H, Song X, Yang S: Development of a RecE/T-assisted CRISPR-Cas9 toolbox for *Lactobacillus*. *Biotechnol J*. 2019, 14[7]:1800690.
<https://doi.org/10.1002/biot.201800690>
61. Kim T-G, Yang M-S: Current trends in edible vaccine development using transgenic plants. *Biotechnol Bioproc*. 2010, 15:61-65.
<https://doi.org/10.1007/s12257-009-3084-2>
62. Kurup VM, Thomas J: Edible vaccines: promises and challenges. *Mol Biotechnol*. 2020, 62[2]:79-90.
<https://doi.org/10.1007/s12033-019-00222-1>
63. William S: A review of the progression of transgenic plants used to produce plant bodies for human usage. *J Young Invest*. 2002;4:56-61.
64. Börner RA, Kandasamy V, Axelsen AM, Nielsen AT, Bosma EF: Genome editing of lactic acid bacteria: opportunities for food, feed, pharma and biotech. *FEMS Microbiol Lett*. 2019, 366[1]:fny291.
<https://doi.org/10.1093/femsle/fny291>
65. Van Zyl WF, Dicks LM, Deane SM: Development of a novel selection/counter-selection system for chromosomal gene integrations and deletions in lactic acid bacteria. *BMC Mol Biol*. 2019, 20.19-11:
<https://doi.org/10.1186/s12867-019-0127-x>
66. Levit R, Cortes-Perez NG, de Moreno de Leblanc A, Loiseau J, Aucouturier A, Langella P, LeBlanc JG, Bermúdez-Humarán LG: Use of genetically modified lactic acid bacteria and bifidobacteria as live delivery vectors for human and animal health. *Gut Microbes* 2022, 14[1]:2110821.
<https://doi.org/10.1080/19490976.2022.2110821>
67. Morello E, Bermudez-Humaran L, Llull D, Sole V, Miraglio N, Langella P, Poquet I: *Lactococcus lactis*, an efficient cell factory for recombinant protein production and secretion. *J Mol Microbiol Biotechnol*. 2008, 14[1-3]:48-58.
<https://doi.org/10.1159/000106082>
68. Benbouziane B, Ribelles P, Aubry C, Martin R, Kharrat P, Riazi A, Langella P, Bermúdez-Humarán LG: Development of a Stress-Inducible Controlled Expression [SICE] system in *Lactococcus lactis* for the production and delivery of therapeutic molecules at mucosal surfaces. *J Biotechnol*. 2013, 168[2]:120-129.
<https://doi.org/10.1016/j.jbiotec.2013.04.019>
69. Mierau I, Kleerebezem M: 10 years of the nisin-controlled gene expression system [NICE] in *Lactococcus lactis*. *Appl Microbiol Biotechnol*. 2005.11-12:1-12:
<https://doi.org/10.1007/s00253-005-0107-6>
70. Nguyen T-T, Nguyen T-H, Maischberger T, Schmelzer P, Mathiesen G, Eijssink VG, Haltrich D, Peterbauer CK: Quantitative transcript analysis of the inducible expression system pSIP: comparison of the overexpression of *Lactobacillus* spp. β -galactosidases in *Lactobacillus plantarum*. *Microb Cell Fact*. 2011, 10:1-10.
<https://doi.org/10.1186/1475-2859-10-46>
71. Cho SW, Yim J, Seo SW: Engineering tools for the development of recombinant lactic acid bacteria. *Biotechnol J*. 2020, 15[6]:1900344.
<https://doi.org/10.1002/biot.201900344>
72. Tavares LM, De Jesus LC, Da Silva TF, Barroso FA, Batista VL, Coelho-Rocha ND, Azevedo V, Drumond MM, Mancha-Agresti P: Novel strategies for efficient production and delivery of live biotherapeutics and biotechnological uses of *Lactococcus lactis*: the lactic acid bacterium model. *Front Bioeng Biotechnol*. 2020, 8:517166.
<https://doi.org/10.3389/fbioe.2020.517166>
73. Yin J-Y, Guo C-Q, Wang Z, Yu M-L, Gao S, Bukhari SM, Tang L-J, Xu Y-G, Li Y-J: Directed chromosomal integration and expression of porcine rotavirus outer capsid protein VP 4 in *Lactobacillus casei* ATCC393. *Appl Microbiol Biotechnol*. 2016, 100:9593-9604.
<https://doi.org/10.1007/s00253-016-7779-y>
74. Goh YJ, Barrangou R: Harnessing CRISPR-Cas systems for precision engineering of designer probiotic lactobacilli. *Curr Opin Biotechnol*. 2019, 56:163-171.
<https://doi.org/10.1016/j.copbio.2018.11.009>
75. Liu L, Helal SE, Peng N: CRISPR-Cas-based engineering of probiotics. *Biodes Res*. 2023, 5:0017.
<https://doi.org/10.34133/bdr.0017>
76. Mougiakos I, Bosma EF, de Vos WM, van Kranenburg R, van der Oost J: Next generation prokaryotic engineering: the CRISPR-Cas toolkit. *Trends Biotechnol*. 2016, 34[7]:575-587.
<https://doi.org/10.1016/j.tibtech.2016.02.004>
77. Westra ER, Van Houte S, Gandon S, Whitaker R: The ecology and evolution of microbial CRISPR-Cas adaptive immune systems. *Philos Trans R Soc Lond B Biol Sci*. 2019;374[1772]:20190101.
<https://doi.org/10.1098/rstb.2019.0101>
78. Pan M, Nethery MA, Hidalgo-Cantabrana C, Barrangou R: Comprehensive mining and characterization of CRISPR-Cas systems in *Bifidobacterium*. *Microorganisms*. 2020, 8[5]:720.
<https://doi.org/10.3390/microorganisms8050720>
79. Jiang Y, Chen B, Duan C, Sun B, Yang J, Yang S: Multigene editing in the *Escherichia coli* genome via the CRISPR-Cas9 system. *Appl Environ Microbiol*. 2015, 81[7]:2506-2514.
<https://doi.org/10.1128/AEM.04023-14>
80. Klompe SE, Vo PL, Halpin-Healy TS, Sternberg SH: Transposon-encoded CRISPR-Cas systems direct RNA-guided DNA integration. *Nature*. 2019, 571[7764]:219-225.
<https://doi.org/10.1038/s41586-019-1323-z>
81. Liu L, Yang D, Zhang Z, Liu T, Hu G, He M, Zhao S, Peng N: High-efficiency genome editing based on endogenous CRISPR-Cas system enhances cell growth and lactic acid production in *Pediococcus acidilactici*. *Appl Environ Microbiol*. 2021, 87[20]:e00948-00921.
<https://doi.org/10.1128/AEM.00948-21>
82. Song X, Huang H, Xiong Z, Ai L, Yang S: CRISPR-Cas9D10A nickase-assisted genome editing in *Lactobacillus casei*. *Appl Environ Microbiol*. 2017, 83[22]:e01259-01217.
<https://doi.org/10.1128/AEM.01259-17>

83. Leenay RT, Vento JM, Shah M, Martino ME, Leulier F, Beisel CL: Genome editing with CRISPR-Cas9 in *Lactobacillus plantarum* revealed that editing outcomes can vary across strains and between methods. *Biotechnol J*. 2019, 14[3]:1700583.
<https://doi.org/10.1002/biot.201700583>
84. Goh YJ, Barrangou R: Portable CRISPR-Cas9N system for flexible genome engineering in *Lactobacillus acidophilus*, *Lactobacillus gasseri* and *Lactobacillus paracasei*. *Appl Environ Microbiol*. 2021, 87[6]:e02669-02620.
<https://doi.org/10.1128/AEM.02669-20>
85. Zhang J, Zong W, Hong W, Zhang Z-T, Wang Y: Exploiting endogenous CRISPR-Cas system for multiplex genome editing in *Clostridium tyrobutyricum* and engineer the strain for high-level butanol production. *Metab Eng*. 2018, 47:49-59.
<https://doi.org/10.1016/j.ymben.2018.03.007>
86. Zhou D, Jiang Z, Pang Q, Zhu Y, Wang Q, Qi Q: CRISPR/Cas9-assisted seamless genome editing in *Lactobacillus plantarum* and its application in N-acetylglucosamine production. *Appl Environ Microbiol*. 2019, 85[21]:e01367-01319.
<https://doi.org/10.1128/AEM.01367-19>
87. Plagens A, Richter H, Charpentier E, Randau L: DNA and RNA interference mechanisms by CRISPR-Cas surveillance complexes. *FEMS Microbiol Rev*. 2015, 39[3]:442-463.
<https://doi.org/10.1093/femsre/fuv019>
88. Bober JR, Beisel CL, Nair NU: Synthetic biology approaches to engineer probiotics and members of the human microbiota for biomedical applications. *Annu Rev Biomed Eng*. 2018, 20[1]:277-300.
<https://doi.org/10.1146/annurev-bioeng-062117-121019>
89. Ramarao N, Lereclus D: Adhesion and cytotoxicity of *Bacillus cereus* and *Bacillus thuringiensis* to epithelial cells are FlhA and PlcR dependent, respectively. *Microbes Infect*. 2006, 8[6]:1483-1491.
<https://doi.org/10.1016/j.micinf.2006.01.005>
90. Ascón MA, Ochoa-Repáraz J, Walters N, Pascual DW: Partially assembled K99 fimbriae are required for protection. *Infect Immun*. 2005, 73[11]:7274-7280.
<https://doi.org/10.1128/IAI.73.11.7274-7280.2005>
91. Sheehan VM, Sleator RD, Fitzgerald GF, Hill C: Heterologous expression of BetL, a betaine uptake system, enhances the stress tolerance of *Lactobacillus salivarius* UCC118. *Appl Environ Microbiol*. 2006, 72[3]:2170-2177.
<https://doi.org/10.1128/AEM.72.3.2170-2177.2006>
92. Sheehan VM, Sleator RD, Hill C, Fitzgerald GF: Improving gastric transit, gastrointestinal persistence and therapeutic efficacy of the probiotic strain *Bifidobacterium breve* UCC2003. *Microbiology*. 2007, 153[10]:3563-3571.
<https://doi.org/10.1099/mic.0.2007/006510-0>
93. Paton AW, Morona R, Paton JC: Designer probiotics for prevention of enteric infections. *Nat Rev Microbiol*. 2006, 4[3]:193-200.
<https://doi.org/10.1038/nrmicro1349>
94. Paton AW, Morona R, Paton JC: A new biological agent for treatment of Shiga toxigenic *Escherichia coli* infections and dysentery in humans. *Nat Med*. 2000, 6[3]:265-270.
<https://doi.org/10.1038/73111>
95. Focareta A, Paton JC, Morona R, Cook J, Paton AW: A recombinant probiotic for treatment and prevention of cholera. *Gastroenterology*. 2006, 1. ١٩٩٥-١٩٨٨:[٩]٣٠.
<https://doi.org/10.1053/j.gastro.2006.02.005>
96. Duan F, March JC: Engineered bacterial communication prevents *Vibrio cholerae* virulence in an infant mouse model. *Proceedings of the National Academy of Sciences Proc Natl Acad Sci U S A*. 2010, 107[25]:11260-11264.
<https://doi.org/10.1073/pnas.1001294107>
97. Lopez-Siles M, Khan TM, Duncan SH, Harmsen HJ, Garcia-Gil LJ, Flint HJ: Cultured representatives of two major phylogroups of human colonic *Faecalibacterium prausnitzii* can utilize pectin, uronic acids and host-derived substrates for growth. *Appl Environ Microbiol*. 2012, 78[2]:420.٤٢٨-
<https://doi.org/10.1128/AEM.06858-11>
98. Shirai M, Pendleton CD, Ahlers J, Takeshita T, Newman M, Berzofsky JA: Helper-cytotoxic T lymphocyte [CTL] determinant linkage required for priming of anti-HIV CD8+ CTL in vivo with peptide vaccine constructs. *J Immunol*. 1994;152[2]:549-556.
99. Oggioni MR, Medaglini D, Romano L, Peruzzi F, Maggi T, Lozzi L, Bracci L, Zazzi M, Manca F, Valensin PE: Antigenicity and immunogenicity of the V3 domain of HIV type 1 glycoprotein 120 expressed on the surface of *Streptococcus gordonii*. *AIDS Res Hum Retroviruses*. 1999, 15[5]:451-459.
<https://doi.org/10.1089/088922299311204>
100. Zegers N, Kluter E, van Der Stap H, Van Dura E, Van Dalen P, Shaw M and, Baillie L: Expression of the protective antigen of *Bacillus anthracis* by *Lactobacillus casei*: towards the development of an oral vaccine against anthrax. *J Appl Microbiol*. 1999, 87[2]:309-314.
<https://doi.org/10.1046/j.1365-2672.1999.00900.x>
101. Villena J, Medina M, Racedo S, Alvarez S: Resistance of Young Mice to Pneumococcal Infection can be Improved by Oral Vaccination with Recombinant *Lactococcus lactis*. *J Microbiol Immunol Infect*. 2010, 43[1]:1-10.
[https://doi.org/10.1016/S1684-1182\[10\]60001-1](https://doi.org/10.1016/S1684-1182[10]60001-1)
102. Buddenborg C, Daudel D, Liebrecht S, Greune L, Humberg V, Schmidt MA: Development of a tripartite vector system for live oral immunization using a gram-negative probiotic carrier. *Int J Med Microbiol*. 2008, 298[1-2]:105-114.
<https://doi.org/10.1016/j.ijmm.2007.08.008>
103. Lee SF: Oral colonization and immune responses to *Streptococcus gordonii*: potential use as a vector to induce antibodies against respiratory pathogens. *Curr Opin Infect Dis*. 2003, 16[3]:231-235.
<https://doi.org/10.1097/00001432-200306000-00008>
104. Wang M, Fu T, Hao J, Li L, Tian M, Jin N, Ren L, Li C: A recombinant *Lactobacillus plantarum* strain expressing the spike protein of SARS-CoV-2. *Int J Biol Macromol*. 2020 .٧٤٠-١٩٠:٧٣٩
<https://doi.org/10.1016/j.ijbiomac.2020.05.239>
105. Fredriksen L, Mathiesen G, Sioud M, Eijsink VG: Cell wall anchoring of the 37-kilodalton oncofetal antigen by *Lactobacillus plantarum* for mucosal cancer vaccine delivery. *Appl Environ Microbiol*. 2010, 76[21]:7359-7362.
<https://doi.org/10.1128/AEM.01031-10>
106. Agarwal P, Khatri P, Billack B, Low W-K, Shao J: Oral delivery of glucagon like peptide-1 by a recombinant *Lactococcus lactis*. *Pharm Res*. 2014, 31:3404-3414.

<https://doi.org/10.1007/s11095-014-1430-3>

107. Durrer KE, Allen MS, Hunt von Herbing I: Genetically engineered probiotic for the treatment of phenylketonuria [PKU]; assessment of a novel treatment in vitro and in the PAHenu2 mouse model of PKU. *PloS one* 2017, 12[5]:e0176286.
<https://doi.org/10.1371/journal.pone.0176286>
108. Wong CC, Zhang L, Wu WK, Shen J, Chan RL, Lu L, Hu W, Li MX, Li LF, Ren SX: Cathelicidin-encoding *Lactococcus lactis* promotes mucosal repair in murine experimental colitis. *J Gastroenterol Hepatol.* 2017, 32[3]:609-619.
<https://doi.org/10.1111/jgh.13499>
109. Jing H, Yong L, Haiyan L, Yanjun M, Yun X, Yu Z, Taiming L, Rongyue C, Liang J, Jie W: Oral administration of *Lactococcus lactis* delivered heat shock protein 65 attenuates atherosclerosis in low-density lipoprotein receptor-deficient mice. *Vaccine* 2011, 29[24]:4102-4109.
<https://doi.org/10.1016/j.vaccine.2011.03.105>
110. Wang X, Chen W, Tian Y, Mao Q, Lv X, Shang M, Li X, Yu X, Huang Y: Surface display of *Clonorchis sinensis* enolase on *Bacillus subtilis* spores potentializes an oral vaccine candidate. *Vaccine* 2014, 32[12]:1338-1345.
<https://doi.org/10.1016/j.vaccine.2014.01.039>
111. Chen T, Tian P, Huang Z, Zhao X, Wang H, Xia C, Wang L, Wei H: Engineered commensal bacteria prevent systemic inflammation-induced memory impairment and amyloidogenesis via producing GLP-1. *Appl Microbiol Biotechnol.* 2018, 102:7565-7575.
<https://doi.org/10.1007/s00253-018-9155-6>
112. Hwang IY, Koh E, Wong A, March JC, Bentley WE, Lee YS, Chang MW: Engineered probiotic *Escherichia coli* can eliminate and prevent *Pseudomonas aeruginosa* gut infection in animal models. *Nat Commun.* 2017, 8[1]:15028.
<https://doi.org/10.1038/ncomms15028>
113. Scott BM, Gutierrez-Vazquez C, Sanmarco LM, da Silva Pereira JA, Li Z, Plasencia A, Hewson P, Cox LM, O'Brien M, Chen SK: Self-tunable engineered yeast probiotics for the treatment of inflammatory bowel disease. *Nat Med.* 2021, 27[7]:1212-1222.
<https://doi.org/10.1038/s41591-021-01390-x>
114. Zhang H, Chen B, Wang Z, Peng K, Liu Y, Wang Z: Resensitizing tigecycline-and colistin-resistant *Escherichia coli* using an engineered conjugative CRISPR/Cas9 system. *Microbiol Spectr.* 2024, 12[4]:e03884-03823.
<https://doi.org/10.1128/spectrum.03884-23>
115. Neil K, Allard N, Roy P, Grenier F, Menendez A, Burrus V, Rodrigue S: High-efficiency delivery of CRISPR-Cas9 by engineered probiotics enables precise microbiome editing. *Mol Syst Biol.* 2021, 17[10]:e10335.
<https://doi.org/10.15252/msb.202110335>
116. Ou B, Jiang B, Jin D, Yang Y, Zhang M, Zhang D, Zhao H, Xu M, Song H, Wu W: Engineered recombinant *Escherichia coli* probiotic strains integrated with F4 and F18 fimbriae cluster genes in the chromosome and their assessment of immunogenic efficacy in vivo. *ACS Synth Biol.* 2020, 9[2]:412-426.
<https://doi.org/10.1021/acssynbio.9b00430>
117. Wang L, Cheng X, Bai L, Gao M, Kang G, Cao X, Huang H: Positive interventional effect of engineered butyrate-producing bacteria on metabolic disorders and intestinal flora disruption in obese mice. *Microbiol Spectr.* 2022, 10[2]:e01147-01121.
<https://doi.org/10.1128/spectrum.01147-21>
118. Ninyio N, Schmitt K, Sergon G, Nilsson C andersson S, Scherbak N: Stable expression of HIV-1 MPER extended epitope on the surface of the recombinant probiotic bacteria *Escherichia coli* Nissle 1917 using CRISPR/Cas9. *Microb Cell Fact.* 2024, 23[1]:39.
<https://doi.org/10.1186/s12934-023-02290-0>
119. Bagherpour G, Ghasemi H, Zand B, Zarei N, Roohvand F, Ardakani EM, Azizi M, Khalaj V: Oral administration of recombinant *Saccharomyces boulardii* expressing ovalbumin-CPE fusion protein induces antibody response in mice. *Front Microbiol.* 2018, 9:723.
<https://doi.org/10.3389/fmicb.2018.00723>
120. Zhang Y, Bailey TS, Hittmeyer P, Dubois LJ, Theys J, Lambin P: Multiplex genetic manipulations in *Clostridium butyricum* and *Clostridium sporogenes* to secrete recombinant antigen proteins for oral-spore vaccination. *Microb Cell Fact.* 2024, 23[1]:119.
<https://doi.org/10.1186/s12934-024-02389-y>
121. Li F, Zhao H, Sui L, Yin F, Liu X, Guo G, Li J, Jiang Y, Cui W, Shan Z: Assessing immunogenicity of CRISPR-NCas9 engineered strain against porcine epidemic diarrhea virus. *Appl Microbiol Biotechnol.* 2024, 108[1]:248.
<https://doi.org/10.1007/s00253-023-12989-0>
122. Valdez Y, Brown EM, Finlay BB: Influence of the microbiota on vaccine effectiveness. *Trends Immunol.* 2014, 35[11]:526-537.
<https://doi.org/10.1016/j.it.2014.07.003>
123. Rhee JH, Lee SE, Kim SY: Mucosal vaccine adjuvants update. *Clin Exp Vaccine Res.* 2012, 1[1]:50-63.
<https://doi.org/10.7774/cevr.2012.1.1.50>
124. Ensign LM, Cone R, Hanes J: Oral drug delivery with polymeric nanoparticles: the gastrointestinal mucus barriers. *Adv Drug Deliv Rev.* 2012, 64[6]:557-570.
<https://doi.org/10.1016/j.addr.2011.12.009>
125. Zahedipour F, Zahedipour F, Zamani P, Jaafari MR, Sahebkar A: Harnessing CRISPR technology for viral therapeutics and vaccines: from preclinical studies to clinical applications. *Virus Res.* 2024, 341:199314.
<https://doi.org/10.1016/j.virusres.2024.199314>

پیشرفت‌های اخیر در واکسن‌های پروبیوتیک خوراکی مهندسی شده: عوامل امیدوارکننده در اثربخشی پروبیوتیک‌ها

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تاریخچه مقاله

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- واکسن‌های خوراکی
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- ویرایش ژنوم CRISPR-Cas9
- انتقال واکسن خوراکی

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چکیده

سابقه و هدف: ارتباط بین پروبیوتیک‌ها به عنوان مکمل‌های غذایی برای بهبود سلامت، مدیریت بیماری و سلامت انسان تاریخچه‌ای طولانی دارد. در حال حاضر پروبیوتیک‌ها دسته بالقوه‌ای از میکروارگانیسم‌ها هستند که در ساخت واکسن‌های خوراکی برای درمان آلرژی، بیماری‌های عفونی و سرطان استفاده می‌شوند. پتانسیل واکسن‌های خوراکی به عنوان واکسن‌های ایمن در برابر عوامل بیماری‌زای احتمالی، تحریک خوب سیستم ایمنی مخاطی و سیستماتیک و هزینه مناسب به دلیل عدم وجود فرآیندهای پیچیده تصفیه، مورد تأکید قرار گرفته است.

یافته‌ها و نتیجه‌گیری: علی‌رغم مزایای پروبیوتیک‌ها به عنوان واکسن‌های خوراکی، استفاده از آنها همچنان با مشکلاتی مانند ناکافی بودن کلونیزاسیون هدفمند، کاهش پاسخ ایمنی در جمعیت‌هایی با استانداردهای پایین بهداشتی، اتکا به میکروبیوتای فردی، پایداری ضعیف، کارایی محدود و نبود ایمنی‌زایی هدفمند روبرو می‌باشد. برای رفع این مشکلات، می‌توان پروبیوتیک‌ها را با استفاده از فناوری‌های اصلاح یا ویرایش ژن، به ویژه سیستم CRISPR/Cas، مهندسی کرد. هنگام استفاده از پروبیوتیک‌های اصلاح‌شده به عنوان واکسن‌های خوراکی، نگرانی‌هایی در مورد ایمنی تغییرات ژنتیکی و کمبود در مکانیسم‌های انتقال کارآمد گزارش شده است. بنابراین، مطالعات بیشتری برای ارزیابی مشکلات مرتبط با تغییر یا اصلاح ژنتیکی دقیق و روش‌های انتقال کارآمد برای دستیابی به هدف نهایی واکسیناسیون‌های مؤثرتر و ایمن‌تر مورد نیاز است.

تعارض منافع: نویسندگان اعلام می‌کنند که هیچ نوع تعارض منافعی مرتبط با انتشار این مقاله ندارند.