International Journal of Nanomedicine Dovepress

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# Engineering Probiotics for Diabetes Management: Advances, Challenges, and Future Directions in Translational Microbiology

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**Background:** Diabetes Mellitus (DM) is a substantial health concern worldwide, and its incidence is progressively escalating. Conventional pharmacological interventions frequently entail undesirable side effects, and while probiotics offer benefits, they are hindered by constraints such as diminished stability and effectiveness within the gastrointestinal milieu. Given these complications, the advent of bioengineered probiotics is a promising alternative for DM management.

**Aim of Review:** The objective of this review is to provide an exhaustive synthesis of the most recent studies on the use of engineered probiotics in the management of DM. This study aimed to clarify the mechanisms through which these probiotics function, evaluate their clinical effectiveness, and enhance public awareness of their prospective advantages in the treatment of DM.

**Key Scientific Concepts of Review:** Scholarly critiques have explored diverse methodologies of probiotic engineering, including physical alteration, bioenrichment, and genetic manipulation. These techniques augment the therapeutic potency of probiotics by ameliorating gut microbiota, fortifying the intestinal barrier, modulating metabolic pathways, and regulating immune responses. Such advancements have established engineered probiotics as a credible therapeutic strategy for DM, potentially providing enhanced results compared to conventional treatments.

**Keywords:** engineered probiotics, diabetes mellitus, gut microbiota, genetic engineering, therapeutic efficacy

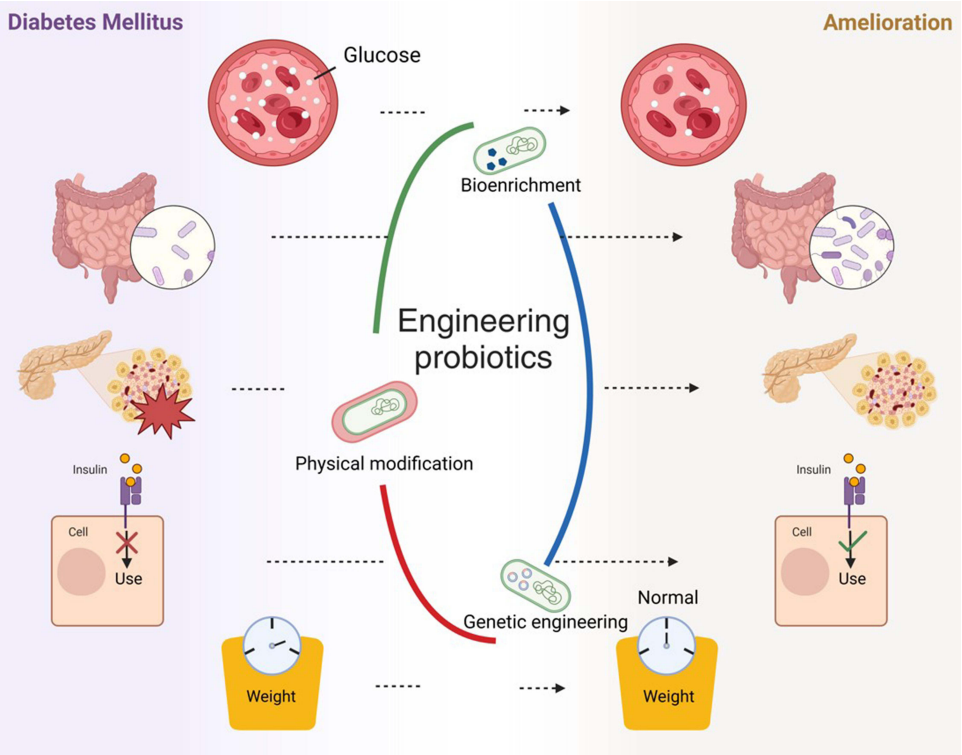
## Introduction

Diabetes mellitus (DM) is a chronic disease characterized by elevated blood glucose levels owing to inadequate insulin secretion or ineffective insulin action.[1] Globally, DM is a major public health concern, with significant impacts on individuals, health systems, and national economies owing to its high prevalence and numerous complications.[2,3] According to the International Diabetes Federation, as of 2021, 537 million adults are affected by DM, with three-quarters residing in low - and middle-income countries, leading to an estimated health expenditure of at least US$ 966.[4] This burden is projected to increase, with 783.2 million people expected to be affected by 2045.[5]

There are two primary types of DM: type 1 diabetes mellitus (T1DM) and type 2 diabetes mellitus (T2DM).[1] T1DM, which mainly affects children and adolescents, is caused by autoimmune destruction of islet β cells, leading to insufficient insulin secretion.[6] Daily insulin injections are the standard treatment. In contrast, T2DM, which can occur

Received: 22 August 2024
Accepted: 8 October 2024
Published: 28 October 2024

**Graphical Abstract**




at any age, is becoming increasingly prevalent in children and adolescents.[7] It is characterized by insulin resistance (IR) and varying degrees of insulin deficiency. T2DM management involves lifestyle interventions and sometimes medications.[8] However, the increasing number of DM cases underscores the inadequacies of the current treatments, which often have side effects. For instance, oral insulin is degraded by the gastrointestinal tract, which reduces its efficacy, and subcutaneous injections pose risks. Metformin, a commonly prescribed medication, can cause adverse reactions such as diarrhea.[9] New drugs such as liraglutide and semaglutide also have side effects including nausea and cardiovascular issues.[10–12] Therefore, there is an urgent need for novel drugs with fewer side effects and more effective delivery systems.

Emerging research has highlighted the relationship between DM and gut microbiota. Disruptions in intestinal flora, particularly a decrease in butyrate-producing species, have been associated with DM development.[13–15] Therefore, improving intestinal flora is a potential therapeutic strategy for DM.[16,17] Probiotics have historically been beneficial for enhancing the intestinal flora.[18] Probiotics, defined as live microorganisms that confer health benefits when administered in adequate amounts, have shown promise in this area.[19–21] Probiotics also possess antioxidant,[22] anti-inflammatory,[23] and metabolic regulatory properties, making them promising candidates for T1DM and T2DM treatments.[24,25] However, orally administered probiotics face challenges, such as reduced survival rates in the gastroenteric environment.[26]

To enhance their efficacy, engineering of probiotics through physical modification, bioenrichment, and genetic engineering is crucial. Physical modifications such as microencapsulation using spray drying and extrusion improve the resilience and survival of probiotics in the digestive tract.[27,28] Bioenrichment involves enriching probiotics with trace elements that enhance their metabolic functions and disease-treatment capabilities.[29,30] Genetic engineering allows for the incorporation of therapeutic genes into probiotics, enabling targeted drug delivery and improving bioavailability.[31]

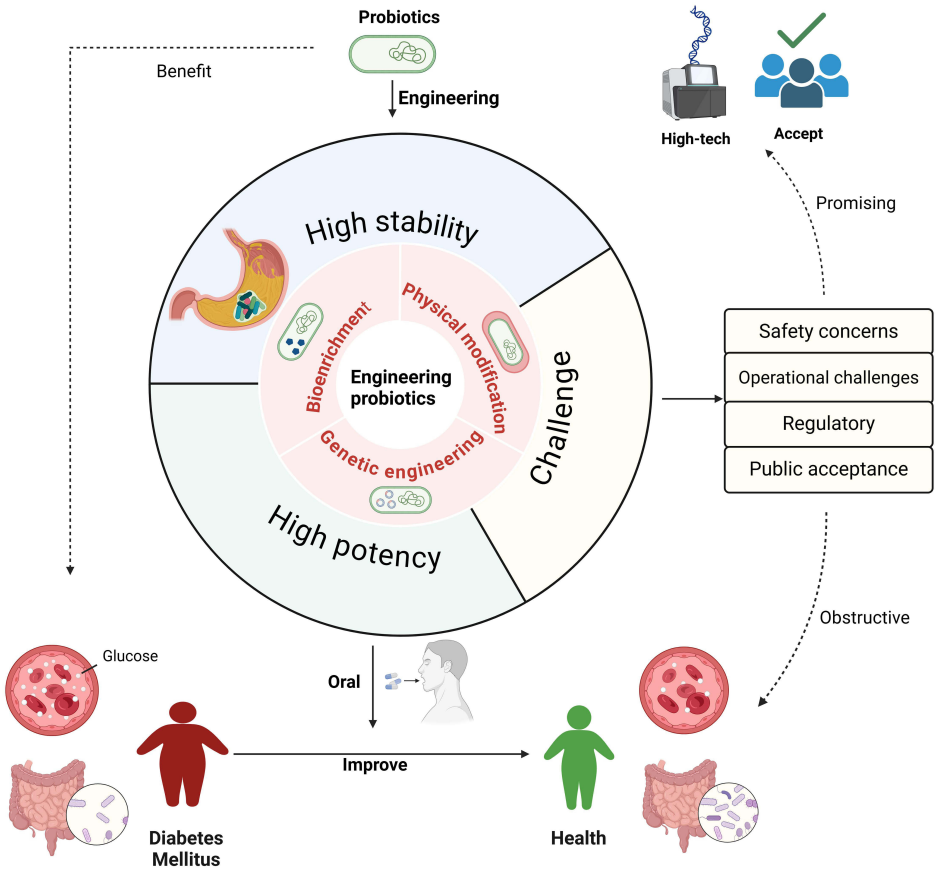



**Figure 1** Engineered probiotics have higher stability and therapeutic efficacy, but face some challenges needed to be solved.

This review discusses the complex interactions between DM and the gut microbiota, design principles and strategies for engineering probiotics, challenges and innovative directions in clinical transformation, and prospects and challenges of engineered probiotics in DM treatment. This study aimed to provide valuable insights for researchers in pharmacy and medicine and to raise public awareness about the potential of engineered probiotics in DM therapy (Figure 1).

## Complex Interactions Between DM and Gut Microbiota

The human gut hosts hundreds of different species of microorganisms, predominantly *Firmicutes, Bacteroidetes, Proteobacteria* and *Actinobacteria. Firmicutes* and *Bacteroidetes* are the most abundant, accounting for 60–80% and 20–30% of gut microbiota, respectively.[32] These microorganisms maintain mutually beneficial relationships with the host and participate in various metabolic processes. Disturbances in gut microbiota can significantly affect the host.[33,34] Alterations in intestinal flora can influence metabolic processes, the intestinal barrier, and the immune response. Probiotics can alleviate DM by improving intestinal flora, enhancing the intestinal barrier, affecting metabolic pathways, and regulating the immune response, thereby contributing to DM alleviation.[35] Probiotics can alleviate DM through the same pathway.[36]

### Relationship Between DM and Gut Microbiota

Extensive research has indicated a close relationship between the gut microbiota and the occurrence of DM. Individuals with low microbial diversity in their gut are more likely to exhibit obesity, dyslipidemia, IR, and a pronounced inflammatory phenotype.[37] The gut microbiota has been shown to affect the intestinal barrier,[38] which in turn can induce T1DM[39] and T2DM.[40]

The intestinal barrier comprises tight junctions and a mucus layer,[41] both of which are critical for preventing pathogenic microorganisms from attaching to the intestinal surface and reproducing and invading epithelial cells. Butyrate, a metabolite produced by many intestinal bacteria, promotes the assembly of tight junction,[42] enhances mucin-2 expression, and regulates repair of the gut mucus barrier.[43] Nevertheless, some bacteria such as *Bacteroides* species associated with DM development[15,44] degrade lactate into substances other than butyrate,[45] thereby reducing butyrate production and increasing intestinal permeability. Additionally, bacterial infections and abnormal bacterial colonization can upregulate zonulin, a protein that increases intestinal permeability by affecting the tight junctions.[35,46] Increased intestinal permeability allows bacterial lipopolysaccharides (LPS) to enter the bloodstream, a process known as metabolic endotoxemia, which is proposed to contribute to chronic low-grade inflammation, IR, and metabolic syndrome.[47] Therefore, a reduction in butyrate-producing bacteria and an increase in harmful bacteria likely contribute to the development of DM.

In addition to butyrate and LPS, other gut microbiota metabolites have been linked to DM.[25] Short-chain fatty acids (SCFAs), such as acetic acid, propionic acid, and butyric acid, are the primary products of microbial metabolism of dietary fiber. SCFAs can induce the production of glucagon-like peptide (GLP-1) by stimulating fatty acid receptor 2, thereby promoting insulin production, regulating islet β-cell proliferation, improving IR, and inhibiting inflammation.[48,49] Normal metabolism of SCFAs inhibits DM, whereas a reduction in SCFA-producing bacteria contributes to T2DM by impairing anti-inflammatory responses and islet cell function, decreasing insulin sensitivity, and promoting IR.[40] Additionally, dietary fiber metabolism primarily occurs in the proximal colon, leaving the distal colon flora to metabolize the remaining proteins. While some protein breakdown products are beneficial, others such as branch-chain amino acids, aromatic amino acids, and trimethylamine are associated with T2DM.[25,50]

The interaction between the gut microbiota and immune system also plays a role in T1DM.[51,52] Toll-like receptors (TLRs), which are pattern recognition receptors expressed on immune and non-immune cells, can recognize pathogen-associated molecular patterns in the microbiome and initiate innate immune responses.[53] Diverse microorganisms can promote or inhibit autoimmunity in T1DM through TLRs activations.[54,55] MyD88, an adaptor protein of multiple TLRs, recognizes microbial stimuli and promotes downstream TLRs signaling pathways.[55] Myd88-null non-obese diabetic (NOD) mice are protected from T1DM under conventional conditions because of their beneficial microbial composition, but they have an increased risk of T1DM under germ-free conditions or antibiotic treatment.[56] This suggests that certain beneficial bacteria protect against T1DM, while disturbances or decreased abundance of gut microbiota increases T1DM risk. Moreover, autoimmune T cells show high cross-reactivity with insulin peptides and certain peptides from *Bacteroides* and *Clostridium*,[57] leading to T1DM. In NOD mice, peptides produced by *Parabacteroides* with homology to the insulin β chain have been identified,[58] and microbial mimic peptides from *Leptotrichia goodfellowii* have been shown to activate autoimmune responses.[59]

## Probiotics and DM

Disturbances in gut microbiota have been implicated in the development of DM. Probiotics, initially proposed as a treatment owing to their capacity to ameliorate the microbiome,[18] have demonstrated potential in managing DM in various clinical trials.[60,61] These findings suggest that probiotics can enhance intestinal flora, fortify the intestinal barrier, influence metabolic pathways, and regulate the immune response, thereby ameliorating DM (Figure 2).

Probiotics enhance gut health by adhering to the intestinal mucosa, preventing colonization by pathogenic microorganisms, and by producing antibacterial substances.[63] They secrete specific proteins that facilitate colonization of the intestinal mucosa by health-promoting microflora, thereby creating spatial barriers that inhibit pathogen colonization.[64,65] Furthermore, probiotics can alter the surrounding environment by producing lactic acid, acetic acid, and other substances[66] that are intolerable to many gram-negative bacteria.[67] Some probiotics produce bacteriocins, which are peptides that inhibit other bacteria by pore formation or inhibition of cell wall synthesis, thereby helping probiotics to compete effectively in their niche.[62,68] Additionally, probiotics can enhance the host resistance to pathogenic microorganisms by increasing IgA levels in the intestinal mucosa.[69,70] All these pathways provide advantages for probiotics to colonize themselves, resist harmful bacteria, and ameliorate intestinal flora.

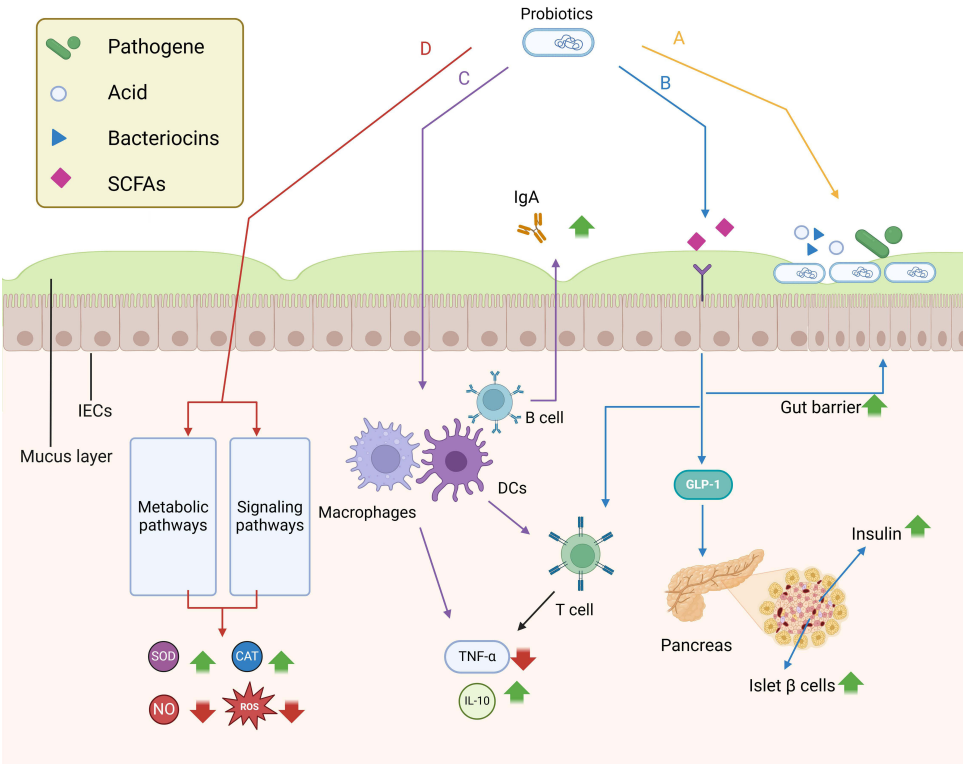



**Figure 2** Mechanism by which probiotics ameliorate DM. A) Probiotics enhance intestinal flora with preventing the invasion of pathogens and secreting bacteriocins or acidic substances.[18] B) probiotics secrete SCFAs, thereby strengthen gut barrier, regulating immune system and producing GLP-1 which protecting the pancreas and promoting insulin secretion.[45] C) Probiotics regulate the immune system, stimulate immune cells to secrete IL-10 and IgA, while reducing TNF-α, and exert anti-inflammatory effects.[62] D) Probiotics possess antioxidant properties, regulating the activities of related factors, superoxide dismutase (SOD), CAT, nitric oxide (NO), and reactive oxygen species (ROS), through metabolic and signaling pathways.[22]

Probiotics also protect the gut by increasing butyrate levels and promoting secretion of intestinal barrier-associated proteins.[71] Butyrate affects the assembly of tight junctions, the synthesis of mucin-2, and the repair of the gut mucus barrier [ref.]. Certain probiotics, such as *Lactobacillus plantarum* (*L. plantarum*), modulate gene transcription pathways related to cell-cell adhesion and enhance tight junction and adhesion junction protein synthesis and degradation by affecting TLR signaling.[72] *Lactobacillus rhamnosus* maintains the epithelial barrier and promotes intestinal epithelial cell activation in response to bacterial infection by enhancing Akt phosphorylation and increasing the expression of Zona occludens-1 and occludin proteins.[73]

Another crucial mechanism by which probiotics ameliorate DM is through their effects on the glucose metabolic pathways.[74] Probiotics enhance SCFA production in humans, thereby increasing insulin secretion, improving IR, and promoting glucose metabolism.[75] In addition, probiotics lower blood glucose levels by regulating oxidative stress and inhibiting carbohydrate hydrolases. Oxidative stress, a significant factor in the induction of T2DM, leads to tissue and organ damage, including islet β-cells, kidneys, and the liver.[76,77] Probiotics possess antioxidant properties; regulate the activities of superoxide dismutase, catalase, and glutathione peroxidase; and inhibit lipid peroxidation and nitric oxide production through metabolic and signaling pathways, thereby reducing oxidative stress markers.[22,78] Furthermore, certain probiotics inhibit the activity of α-glucosidase and α-amylase, influence glucose metabolism, alleviate postprandial hyperglycemia, and regulate blood glucose levels.[79]

The anti-inflammatory effects of probiotics are vital for DM management. There is a strong correlation between inflammation and the T2DM development.[80] Pro-inflammatory factors, such as tumor necrosis factor-α (TNF-α), inhibit the phosphorylation of insulin receptor substrates, negatively affect insulin signaling, and cause IR.[81] Other factors, including adipokines, chemotactic proteins, and interleukin-6 (IL-6), are also associated with DM development.[82] Probiotics impede pathogen invasion and enhance the intestinal flora composition, thereby preventing the onset.[82] The

immunomodulatory effects of probiotics have been shown to ameliorate inflammation and to alleviate DM.[83] For instance, *Lactobacillus acidophilus* KLDS1.0901 improved intestinal barrier function and suppressed inflammatory responses in the liver and colon in animal models of T2DM.[84] Similarly, *Lactobacillus casei* CCFM419 attenuated IR, pancreatic islet impairment, and proinflammatory markers in diabetic mice.[85] Increased expression of TLR2, TLR4, and TLR9, along with improved production and secretion of TNF-α, interferon-γ (IFN-γ), and IL-10 have been observed in mice treated with probiotics.[86] Probiotics also induce naive T cells to differentiate into regulatory T cells (Tregs) and produce high levels of IL-10 and transforming growth factor-β (TGF-β) by activating TLR-2 receptors on macrophages and dendritic cells.[87,88] Additionally, *Lactobacillus reuteri* ATCC PTA 6475 suppressed TNF transcription in monocyte-derived macrophages by inhibiting the activation of mitogen-activated protein kinase-regulated c-Jun and transcription factor activation protein-1.[89] Certain probiotics improve obesity, enhance insulin signaling sensitivity, and ameliorate IR by reducing monocyte chemoattractant protein-1 and preserving natural killer T cells.[90] Probiotics and their metabolites interact with various immune cells to maintain immune homeostasis by balancing the pro-inflammatory and anti-inflammatory responses.[91]

Moreover, the immunomodulatory effects of probiotics are also significant in T1DM treatment. Autoreactive T cells attack islet β-cells, leading to insulitis and reduced β-cell mass, resulting in insulin deficiency, which is a key factor in T1DM pathogenesis.[92] Probiotics alleviate T1DM by reducing insulitis through anti-inflammatory effects.[24] After probiotic intervention, T1DM patients exhibit increased TGF-β1 expression and decreased concentrations of IL-8, IL-17, RANTES (a chemotactic protein), and TNF-α.[93] Oral probiotic administration induces IL-10 production, reduces islet β-cell destruction, and prevents DM.[94] Probiotic metabolites such as primary SCFAs can limit the frequency of autoreactive T cells. Acetate reduces the frequency of autoreactive T cells in lymphoid tissues by influencing islet β cells and expanding the autoreactive T cell population. Butyrate enhances the number and functionality of regulatory T cells.[95]

Although the precise mechanisms by which probiotics treat DM remain unclear, a growing body of evidence, including the aforementioned studies, suggests that probiotics have demonstrated promising results in the clinical management of diabetes, and may be an effective therapeutic option for DM.[61,96] Probiotics have the potential to increase the levels of beneficial substances, enhance intestinal barrier protection, improve intestinal flora, ameliorate IR, reduce blood glucose levels, and exert other potential effects in DM treatment.

## Design Principles and Strategies of Engineered Probiotics

Although probiotics have shown potential in ameliorating DM, their supplementation remains minor compared to that of the resident gut microbiota.[18] The unclear mechanism by which probiotics treat DM, combined with the significant limitations and limited efficacy of natural probiotics, necessitate the development of advanced strategies. Oral probiotics are one of the easiest ways to quickly supplement probiotics; however, probiotics that enter the body are greatly affected by the environment, such as pH, enzymes, and mechanical agitation in the digestive tract, which reduce their activity and effectiveness upon reaching the gut.[28,97] Probiotic engineering is essential to overcome these challenges and enhance therapeutic efficacy. Currently, the most widely used techniques for probiotic engineering include physical modification, bioenrichment, and genetic engineering (Figure 3). Each method has distinct advantages and caters to various requirements. The following sections describe these techniques in detail:

### Physical Modification

Physical modification is one of the most prevalent methods for engineering probiotics, leveraging materials such as alginates to encapsulate and microencapsulate probiotics and to ensure their resistance to the hostile environment of the gastrointestinal tract.[28] These encapsulating materials facilitate the safe delivery of probiotics to their target sites, where they can exert beneficial effects in treating diseases, such as DM. This method has been extensively developed and applied to the treatment of inflammatory bowel disease and DM.[100,101]

A common microencapsulation technique is extrusion, which is straightforward, cost-effective, and allows modified probiotics to retain their optimal cell viability.[27] For instance, Arriaga-Morales et al[98] suspended *Lactobacillus casei subsp. casei* NRRL-1922 in 2% sodium alginate solution. Using a peristaltic pump adapted to the syringe hose, droplets of *Lactobacillus casei* cell suspension in sodium alginate were added to $CaCl_2$ to form calcium alginate gel particles. The

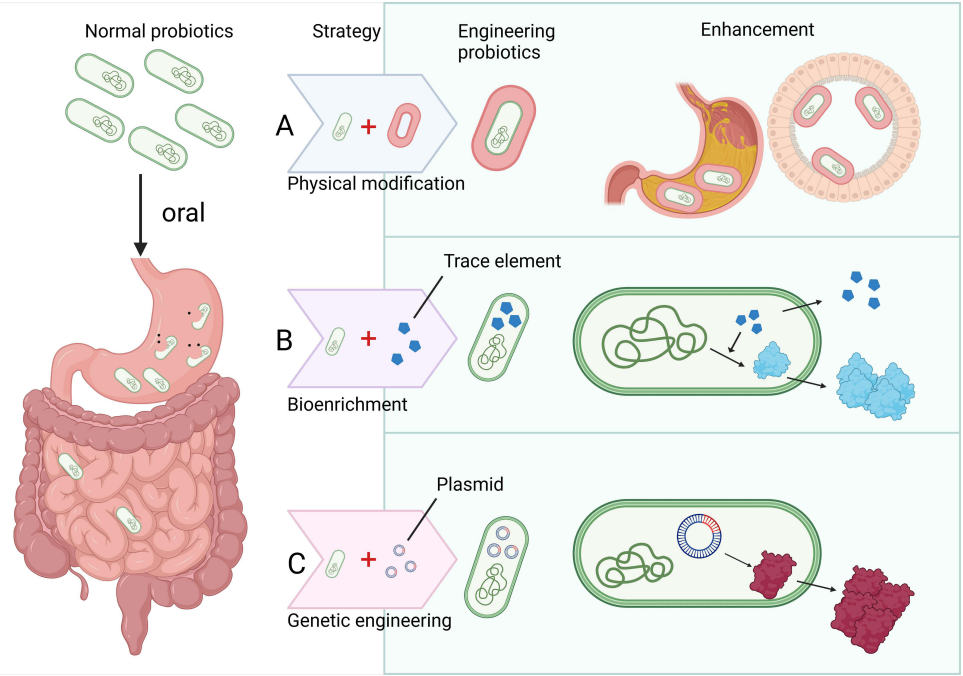



**Figure 3** Three strategies of engineering probiotics and their enhancement. A) Physical modification: Probiotics were coated with special materials to improve their stability and colonization.[98] B) Bioenrichment: Probiotics are enriched by trace elements to enhance the production of beneficial metabolites and facilitate the secretion of trace elements.[29] C) Genetic engineering: Probiotics are transformed by plasmid to secrete medicinal protein.[99]

survival rate of immobilized probiotics was 51.9%, whereas that of free probiotics was 2.1%. Immobilized probiotics showed good hypoglycemic effects in streptozotocin-nicotinamide-induced T2DM rats. Compared to the disease group, the blood glucose reduction rate of the immobilized probiotic treatment group was 70.3%. Compared to free probiotics, the serum glucose concentration of the immobilized probiotic treatment group was reduced by 67.8%. In addition, the immobilized probiotic treatment had protective effects on the kidneys and liver. The results demonstrated that the immobilized probiotics prepared using this technique were more effective than free probiotics in the treatment of DM (Figure 4A).

Encapsulation can include both probiotics and therapeutic substances to enhance the therapeutic effects. For example, Gómez-Fernández et al[102] used a spray-drying method, a more efficient microencapsulation method, spray-dried *L. plantarum* 299v, and *Lactobacillus acidophilus* La3 suspended in 750 mL of a microencapsulation mix (10% w/v maltodextrin and w/v 2% food-grade alginate) at 130 °C inlet, 60 °C outlet, and 0.13 MPa. The microcapsules were then packaged with fish oil (as an additional medicine), using sugar-free milk chocolate, to create a stabilized probiotic product. The obtained probiotic products exhibited high probiotic content ($7 \times 10^{13}$ CFU/g of *L. plantarum* 299v and $1 \times 10^{14}$ CFU/g of *Lactobacillus acidophilus* La3), and the ω-3 polyunsaturated fatty acid content in fish oil was 133.8 ± 8.76 mg per 12 g of probiotic products (Figure 4B).

Lipophilic drugs, such as fish oil, which are effective in DM treatment, face challenges in oral delivery owing to limited dissolution and bioavailability. Self-nanoemulsifying drug delivery systems (SNEDDS) can address this issue. Khursheed et al[103] formulated an optimal ratio of four substances, diethylene glycol monoethyl ether, twin 80, oleoyl polyoxyl-6 glycerides, and mono-diglyceride, which are used to dissolve the lipophilic plant drugs curcumin and quercetin, both of which have been reported to have antidiabetic effects. SD-S-SNEDD was prepared by spray drying S-SNEDDS, which was prepared by ganoderma lucidum extract powder and probiotics as solid supports based on L-SNEDDS. To further improve its physical and mechanical stability, SD-S-SNEDDS was transformed into spherical particles via extrusion spheronization. The result found The zeta potential of S-SNEDDS was −38.7 mV, indicating that S-SNEDDS exhibited better absorption and oxidation stability. The drug loading percentages of curcumin and quercetin in SD-S-SNEDDS were 97.8±2.32% and 96.5±3.17%, respectively, and more than 90% of the drug was released within the first 5 min. Furthermore, SD-S-SNEDDS exhibited excellent absorption and bioavailability and enhanced α-

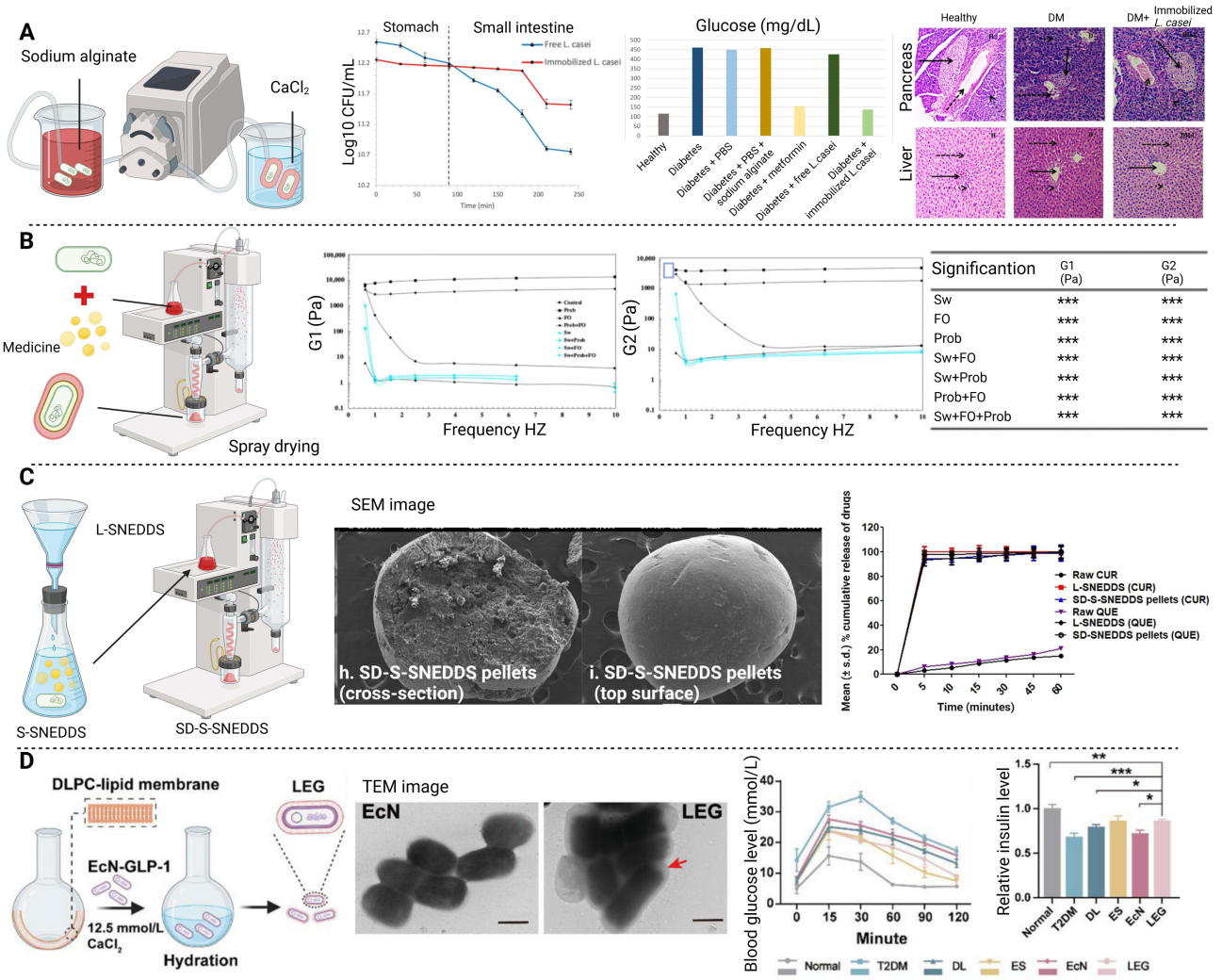



**Figure 4** (**A**) The immobilized Lactobacillus casei, built by extrusion, showed a high survival rate, blood glucose reduction rate and protective effects on the kidney. Reproduced with permission from Arriaga-Morales JJ, Ordaz-Pichardo C, Castro-Muñoz R, Durán-Páramo E. Attenuation of hyperglycemia in diabetic rats assisted by immobilized probiotic in sodium alginate. Probiotics Antimicrob Proteins. 2023.[98] Copyright 2023 Springer Nature. (**B**) Probiotic product, created by spray drying, had great stability. Full factorial analysis of variance showing the main effects and interactions of the variables evaluated. G1 is an index of a sample's elastic behavior and represents the deformation energy stored in the sample during the shear process. G2 value measures the viscous component of a sample and compares the energy lost during the shear process. Control = milk chocolate formulation, Prob = milk chocolate + probiotics, FO = milk chocolate + fish oil, Prob + FO = milk chocolate + probiotics + fish oil, Sw = isomalt + stevia, Sw + Prob = isomalt + stevia + probiotics, Sw + FO = isomalt + stevia + fish oil, Sw + Prob + FO = isomalt + stevia + probiotics + fish oil. Reproduced with permission from Gómez-Fernández AR, Faccinetto-Beltrán P, Orozco-Sánchez NE, et al Sugar-free milk chocolate as a carrier of Omega-3 polyunsaturated fatty acids and probiotics: a potential functional food for the diabetic population. Foods. 2021;10:1866.[102] Copyright 2021 by the authors, licensee MDPI, Basel, Switzerland. (**C**) The process of constructing SD-S-SNEDDS, the SEM image of SD-S-SNEDDS and cumulative release of drugs. Reprinted from International Journal of Pharmaceutics, 612, Rubiya Khursheed, Sachin Kumar Singh, Bimlesh Kumar, Sheetu Wadhwa, Monica Gulati, Anupriya A, Ankit Awasthi, Sukriti Vishwas, Jaskiran Kaur, Leander Corrie, Arya K.R., Rajan Kumar, Niraj Kumar Jha, Piyush Kumar Gupta, Flavia Zacconi, Kamal Dua, Nitin Chitranshi et al, Self-nanoemulsifying composition containing curcumin, quercetin, Ganoderma lucidum extract powder and probiotics for effective treatment of type 2 diabetes mellitus in streptozotocin inducedrats, 121306, Copyright (2024), with permission from Elsevier[103] (**D**) Schematic illustration of the preparation of encapsulating Escherichia coli Nissle 1917, the TEM image of Escherichia coli (EcN) and encapsulated Escherichia coli (LEG) and the blood glucose level and insulin level of T2DM mice after different treatments. Reproduced with permission from Wang Y, Shi Y, Peng X, et al Biochemotaxis-oriented engineering bacteria expressing GLP-1 enhance diabetes therapy by regulating the balance of immune. Adv Healthcare Mater. 2024;13:2303958. © 2024 Wiley-VCH GmbH[104] Asterisks indicate significant difference from a full factorial analysis of variance showing the main effects and interactions of the variables evaluated: *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

glucosidase inhibition. In diabetic rats, SD-S-SNEDDS showed hypoglycemic, lipid-lowering, liver- and kidney-protective effects and a favorable recovery of diabetic parameters (Figure 4C).

Liposomes are popular drug delivery systems in which the phospholipid bilayer provides excellent protection.[105] Moreover, certain phospholipids have been shown to exert adjunctive antidiabetic effects. Recently, Wang et al[104] encapsulated *Escherichia coli* Nissle 1917, which is capable of expressing GLP-1, using a film dispersion method with a dilauroyl phosphatidylcholine-based lipid membrane. Lipid membranes protect probiotics from attack by the gastrointestinal

environment, effectively inhibiting the production of reactive oxygen species, alleviating oxidative stress, and repairing pancreatic islet β-cell dysfunction with GLP-1. Furthermore, the engineered probiotics regulated insulin secretion, upregulated the expression of key proteins, and enhanced the richness and diversity of intestinal flora (Figure 4D).

## Bioenrichment

Trace elements are crucial for the functioning of living organisms including humans and probiotics.[106] Bioenrichment involves enriching trace elements with probiotics to enhance therapeutic efficacy. Introducing trace elements into probiotics has been shown to ameliorate DM independently and to enhance the therapeutic effects of probiotics by strengthening metabolic enzymes or pathways. This approach combines the direct and indirect therapeutic effects of trace elements with those of probiotics to exert a greater influence on the body than their individual effects.

In probiotics, some enzymes or other substances produced by metabolism can improve DM. For example, dihydronicotinamide-adenine dinucleotide and glucuronic acid dehydrogenase in *Acetobacter aceti* (*A. aceti*) mediate the glycolysis pathway, enabling the breakdown of glucose and the subsequent achievement of a hypoglycemic effect. However, the amount of these substances in normal probiotics does not meet the expectations for disease treatment. Huang et al[29] devised a bioenrichment method to enhance the metabolites in probiotics by increasing the quantity of zinc and chromium in probiotics, which in turn enhances the healing capacity of the disease. Notably, zinc, an important component of insulin, can significantly ameliorate glucose tolerance and glucose-stimulated insulin secretion by enhancing the function of islet β-cells, thereby improving and preventing DM.[107,108] Similarly, Cr has been shown to exert beneficial effects on blood glucose control.[109] They obtained chromium- and zinc-rich *A. aceti* after being cultured for a period of time (48 h) in liquid medium containing 64 mg/mL chromium trichloride and zinc chloride. After detection, the content of chromium metal, metallic zinc, NADH coenzyme, and glucose dehydrogenase in *A. aceti* prepared by this method were 28.58–34.34 mg/kg, 5.35–7.52 mg/kg, 5.13–7.26 μM, and 446.812–567.138 U/g, respectively. The levels of these substances were higher than those in uncultured Acetobacter spp. The fasting blood glucose level of diabetic mice treated with the modified probiotics was lower than that of mice treated with metformin tablets. It also protects islet cells, alleviates islet tissue damage, and restores the body weight. It is worth mentioning that mice treated with ten-fold dose of *A. aceti* rich in chromium and zinc did not show pathological damage to the liver, spleen, kidney, or stomach, which was sufficient to prove the safety of the engineered probiotics (Figure 5A).

Selenium (Se), another essential micronutrient, has demonstrated antidiabetic properties by promoting sustained improvement in glucose homeostasis and regulating key metabolic processes such as glycolysis, gluconeogenesis, and lipid metabolism.[112,113] Zhao et al[110] used a similar approach to fermenter *Bifidobacterium longum* DD98 in selenium-rich medium, producing selenium-enriched *Bifidobacterium longum* DD98 (Se-*B. longum* DD98). Oral administration of Se-*B. longum* DD98 in diabetic mice reduced fasting blood glucose levels, glycosylated hemoglobin levels, and pancreatic injury, and improved glucose tolerance, outperforming both untransformed *B. longum* DD98 and $Na_2SeO_3$ treatments (Figure 5B).

Lin et al[111] advanced this approach using selenium nanoparticles. They pretreated selenium with a mixture of sodium selenite, reduced glutathione, and bovine serum albumin to produce red nano-selenium. After dialysis with double-distilled water, magnetic stirring, and centrifugation, the purified selenium nanoparticles were obtained. Bifidobacterium longum NQ-1501 was cultured anaerobically overnight in TPY medium containing 25 μg/mL sodium selenite and 5 μg/mL selenium nanoparticles to obtain Nano-Se-*B. longum*. It is pertinent to note that the selenium in Nano-Se-*B. longum* exists in the form of selenoproteins, which have been documented to exhibit a high bioavailability and low toxicity.[114] Diabetic mice induced with streptozotocin (STZ) treated with Nano-Se-*B. longum* exhibited significantly lower fasting blood glucose levels, higher body weights, and higher fasting insulin levels than the WT *B. longum*. Moreover, Nano-Se-B. longum alleviated pathological damage to the liver, pancreas, and kidney and improved the sensitivity of hepatic insulin signaling (Figure 5C).

## Genetic Engineering

Genetic engineering is a widely used method for modifying probiotics for the treatment of DM. This approach involves the introduction of genes encoding therapeutic proteins into the probiotics. Engineered probiotics can produce and deliver these proteins directly to specific locations in the gastrointestinal tract, thereby enhancing their therapeutic efficacy by protecting them from degradation. Probiotics can also serve as expression vectors to synthesize therapeutic substances,

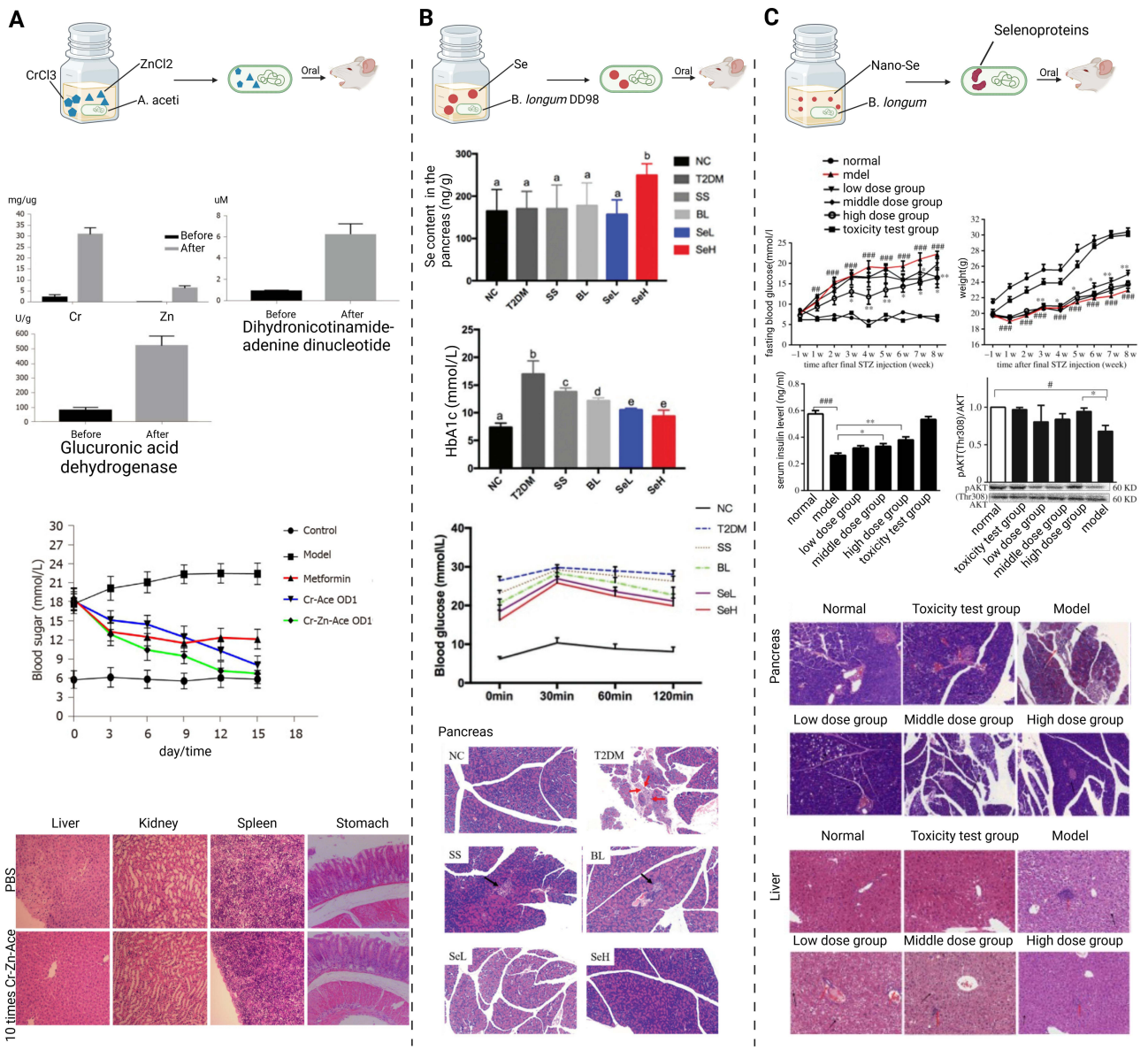



**Figure 5** (**A**) The content of related substances in Cr- and Zn-rich *A. aceti* had been improved, and they had good hypoglycemic effect, and had no damage to many organs. Reproduced with permission from Huang -Y-Y, Qin X-K, Dai -Y-Y, et al Preparation and hypoglycemic effects of chromium- and zinc-rich Acetobacter aceti. World J Diabetes. 2022;13:442–453..[29] Copyright 2022, the Author(s). Published by Baishideng Publishing Group Inc. All rights reserved. (**B**) Se content in the pancreas had been enhanced and the glycated hemoglobin (Hb1Ac), blood glucose level and pancreatic injury had been reduced under the treatment of Se-*B. longum* DD98. NC, normal control group; T2DM, model group; SS, sodium selenite group; BL, (**B**) longum DD98 group; SeL, Se-B. longum DD98 low dosage group; SeH, Se-B. longum DD98 high dosage group. $^{a-e}$Means with different letters differ significantly for the same indicator after different treatments ($p < 0.05$). Used with permission of Royal Society of Chemistry, from Antidiabetic effects of selenium-enriched Bifidobacterium longum DD98 in type 2diabetes model of mice, Zhao D, Zhu H, Gao F, et al, 11, 7, copyright 2010; permission conveyed through Copyright Clearance Center, Inc. [110] (**C**) The content of fasting blood glucose levels, body weights and higher fasting insulin levels, and the expression of insulin signaling pathway proteins had been improved by Nano-Se-*B. longum*. $^{\#}p < 0.05$, $^{\#\#}p < 0.01$, $^{\#\#\#\#}p < 0.001$ compared with normal group; $^{*}p < 0.05$, $^{**}p < 0.01$ compared with the model group. Reproduced with permission from Lin Y, Ren Y, Zhang Y, et al Protective role of nano-selenium-enriched Bifidobacterium longum in delaying the onset of streptozotocin-induced diabetes. R Soc Open Sci. 2018;5:181156.[111] Copyright 2018 The Authors. Published by the Royal Society under the terms of the Creative Commons Attribution License.

making treatment more accessible and cost-effective.[31] Key therapeutic proteins used in this context include insulin and its analogs, GLP-1, GLP-1 receptor agonists (GLP-1RAs), antigens, ILs, and other proteins known to play roles in DM treatment (Table 1).

## Insulin and its Analogues

In 1978, Goeddel et al pioneered the synthesis of the first recombinant human insulin DNA by combining single strands of A and B expressed in *Escherichia coli*.[115] Most insulin produced in this way is suitable for injection rather than oral

**Table 1** The Summary of Major Studies on the Use of Engineering Probiotics in the Management of DM

| Probiotics | Disease models | Drug | Drug Delivery Routes | Contact with Gut Bacteria | Therapeutic Effect | References |
|---|---|---|---|---|---|---|
| *L. plantarum* | T2DM monkeys | GLP-1 | Oral Probiotic Supplement | Substantial reduction in the intestinal pathogen *Prevotella* and marked enhancement of butyrate-producing *Alistipes* genera. | Fasting blood glucose of monkeys was significantly reduced to a normal level and only a small amount of weight was lost. | [111] |
| *Saccharomyces cerevisiae* | STZ and HFD induced T2DM mice | GLP-1 | Oral Probiotic Supplement | | Improved body weight, food ingestion, water consumption and blood glucose of diabetic mice | [114] |
| *Saccharomyces cerevisiae* | STZ induced T2DM mice | GLP-1 | Oral Probiotic Supplement | | Lowered blood sugar, prevented weight loss, control diet and improved water intake | [115] |
| *Lactococcus lactis MG 1363* | HFD-induced obese mice | GLP-1 | Oral Probiotic Supplement | *Proteobacteria* richness decreased significantly, *Firmicutes* richness increased further, intestinal microbial diversity increased, and composition returned to normal level | Reduced body weight and improved glucose intolerance and liver function in obese mice | [116] |
| *Lactobacillus garneri ATCC 33323* | STZ-mediated DM mice | GLP-1 | Oral Probiotic Supplement | | Improved hyperglycemia and increases insulin levels | [117] |
| *Escherichia Coli* BL21 (DE3) | HFD-induced DM mice | 10×rolGLP-1 | Oral expressed product supplement | | Lowered fasting blood glucose, maintained weight, and improved oral glucose tolerance | [118] |
| *Lactobacillus paracei L14* | INS-1 cells | Exendin-4 | In vitro | | Promoted the proliferation of isletβcells, reduced cell apoptosis, promoted insulin secretion and exendin-4 through the Caco-2 cell monolayer | [119] |
| *L. plantarum* | STZ and HFD induced T2DM mice | GLP-1 | Oral Probiotic Supplement | Increased *Lactobacillus, Akkermansia, Clostridium XlVa, Alloprevotella* and *Clostridium* IV and decreased *Odoribacter, Bacteroides, Actetatifactor* and *Desulfovibrio* | Inhibited of islet cell apoptosis, promoted restoration of the islet tissue morphology, the improvement of the insulin secretion and pancreatic inflammation, regulated lipid metabolism in the liver, and improved blood glucose tolerance in mice | [120] |
| *Escherichia Coli* BL21 (DE3) | STZ-induced DM mice | Two, four and eight series of recombinant GLP-1 | Oral expressed product supplement | | Decreased serum glucose levels | [121] |
| *L. lactis* MG1363 | T1DM mice | Proinsulin and IL-10 | Oral probiotics and intravenous anti-CD3 | | Reverted T1DM and increased frequencies of local Tregs | [122] |
| *L. lactis* MG1363 | NOD mice | Proinsulin and IL-10 | Oral probiotics and intravenous anti-CD3 | | Reestablished tolerance to β cells, provided persistence of insulin-containing islets or prolonged βcell function | [123] |
| *L. lactis* MG1363 | T1DM mice | Proinsulin and IL-10 | Oral probiotics and intravenous anti-CD3 | | Reverts new-onset diabetes, preserves residual β-cell function, and halts insulitis progression | [124] |

(*Continued*)


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**Table 1** (Continued).

| Probiotics | Disease models | Drug | Drug Delivery Routes | Contact with Gut Bacteria | Therapeutic Effect | References |
|---|---|---|---|---|---|---|
| *L. lactis* FI5876 | HFD feed mice | GLP-1 | Oral Probiotic Supplement | | Promoted insulin secretion, improved glucose tolerance | [125] |
| *E. coli transetta* (DE 3) | STZ and HFD induced T2DM mice | Recombination 6×mGLP-1 | Intraperitoneally injected expression | | Lowered blood glucose, Stimulated the proliferation of mouse pancreatic β cell line MIN6 in vitro | [126] |
| *Lactococcus lactis NZ9000* | The murine 3T3-L1 preadipocyte cell line | Single chain insulin analog SCI-57 | In vitro | | Had active insulin function | [127] |
| *Escherichia coli BL21* | NOD miec | HSP65-6P277 | Nasal expressed product administration | | Decreased the incidence of diabetes, inhibited insulitis, reduced cytokines IFN-gamma and IL-2 secretion | [128] |
| *L. lactis NZ9000* | NOD miec | HSP65-6IA2P2 | Oral Probiotic Supplement | | Prevented hyperglycemia, improved glucose tolerance, and reduced insulitis by inhibiting antigen-specific proliferation of T cells, augmenting regulatory immune reactions, and balancing ratios of Th17/Tregs and Th1/Th2 | [129] |
| *L. lactis strain NZ9000* | NOD mice | HSP65-6P277 | Oral Probiotic Supplement | | Prevented hyperglycemia, improved glucose tolerance, reduced insulitis and induced HSP65- and P277- specific T cell immuno-tolerance and antigen-specific proliferation of splenocytes | [130] |
| *L. lactis NZ9000* | NOD mice | Staphylococcal nucleases | Oral Probiotic Supplement | | Disrupted neutrophil extracellular traps, ameliorated inflammation, regulated the blood glucose levels, and the onset of diabetes was postponed with reduced mortality and morbidity | [131] |
| *L. lactis* | NOD mice | CFA/I | Oral Probiotic Supplement | Enriched of *Lactobacilli* and *Ruminococcus* | Reduced disease incidence, increased splenic Tregs producing both IL-10 and IFN-γ 8-fold | [132] |
| *L. lactis* | NOD mice | CFA/I | Oral Probiotic Supplement | | Promoted regulatory APC to suppress inflammatory T cell responses and promoted Foxp3−Treg responses, stimulated DCs to establish a regulatory microenvironment | [133] |
| *L. lactis MG 1363 FnBPA+* | NOD mice | IL-4 and IL-10 | Intragastrically administered probiotics | | Prevented hyperglycemia, reduced islet destruction and increased IL-4 and IL-10 levels in serum and pancreatic tissue | [134] |
| *L. lactis NZ9000* | INS-1 cells | Exendin-4 | In vitro | | Enhanced glucose-dependent insulin secretion and activated the PI3-K/AKT signal pathway | [135] |

| | | | | | | |
|---|---|---|---|---|---|---|
| *L. lactis NZ 3900* | Murine 3T3-L1 preadipocytes | Single chain insulin analog SCI-57 bonded with non-viable bacteria(NVBs) | In vitro | | SCI-59 displayed on the surface of NVBs were biologically active and stimulated Akt signaling in differentiated 3T3-L1 adipocytes | [136] |
| *L. lactis NZ 3900* | NOD mice | Single chain insulin analog SCI-57 bonded with bacterium-like particles | Oral Probiotic Supplement | | Prevented T1DM, improved glucose tolerance, reduced insulitis | [99] |
| *L. plantarum* WCFS1 | Db/db mice | ModifiedGLP-1 | Oral Probiotic Supplement | | Improvement of diabetes symptoms associated with decreased glucagon, increased pancreatic beta cell proportion, and increased insulin sensitivity | [137] |
| *L. lactis* NZ 9000 | Male C57BL/6 mice | L-arabinose isomerase | Intragastrically administered probiotics | | Converted the galactose into tagatose, lowered hyperglycemia | [138] |
| *L. lactis MG1363* | NOD mice | Glutamic acid decarboxylase 65 and IL-10 | Oral Probiotic Supplement | | Reversed T1DM, prevented progression of insulitis | [139] |
| *Lactobacillus paracasei BL23* | T2DM rats | 5×GLP-1 | Intra-intestinal administration of the 5×GLP-1 peptide | | Promoted insulin secretion and lowered blood glucose levels | [140] |
| *Escherichia coli* Nissle 1917 | T2DM mice | GLP-1 | Oral Probiotic Supplement | Inhibited the colonization of pathogens, increased the diversity and relative abundance *Enterobacteriales* of *Lactobacillus*, decreased abundance of *Ruminococcaceae* and regulated the balance of intestinal flora | Alleviated oxidative stress, repair pancreatic islet β-cell dysfunction, regulated the insulin secretion, cholesterol metabolism | [103] |



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administration. Owing to the advantages of oral insulin,[116] researchers have focused on expressing insulin in probiotics to simulate oral insulin delivery. *Lactococcus lactis* (*L. lactis*), a safe and well-characterized probiotic, is commonly used for this purpose.[117,118] However, synthesizing insulin, a heterodimeric protein with three disulfide bonds, using *L. lactis* presents specific challenges.

To overcome this challenge, Ng et al[119] expressed the single-chain insulin analog SCI-57 in *L. lactis* ZN9000. SCI-57, designed for oral administration, simplifies synthesis while maintaining biological activity and stability comparable to those of wild-type insulin. They constructed the plasmid pNZPnisA:uspSCI-57his, which was transformed into *L. lactis* strain ZN9000. Recombinant *L. lactis* successfully secreted active SCI-57 under the induction of nisin at certain pH conditions.

Mao et al[120] developed another single-chain insulin, SCI-59, linked by an eight-residue linker between insulin B and A chains. SCI-59 was optimized and introduced into *L. lactis* NZ3900. Rather than using the recombinant bacteria directly, SCI-59-3LysM was combined with non-viable LAB to avoid the use of antibiotic resistance genes and to enhance stability. This engineered probiotic reduces DM incidence, improves glucose tolerance, promotes C-peptide secretion, ameliorates insulitis, and improves the regulatory immune response in NOD mice.[121]

## Glp-1

GLP-1 is a 30-amino acid peptide hormone secreted by intestinal cells that plays a crucial role in glycemic control in patients with T2DM by interacting with the GLP-1 receptor.[122] GLP-1 is rapidly degraded by dipeptidyl peptidase-4 (DPP-4) and trypsin, leading to a short half-life. To address this issue, researchers have explored various strategies to enhance the stability and efficacy of GLP-1 delivered through probiotics.

Hu et al[123] synthesized the plasmid pUC-SPglnPH2-GLP-1 by incorporating the codon-optimized GLP-1 gene with glnPH2 signal peptide sequences (SPglnPH2) in the pUC57 vector. This DNA fragment was PCR-amplified, digested, and ligated into the pMG36e plasmid, which was then electrotransformed into *L. plantarum* to produce *L. plantarum*-pMG36e-GLP-1. In T2DM mice, these engineered bacteria effectively reduced blood glucose levels, improved glucose tolerance, and inhibited weight gain by reducing pancreatic inflammation, improving the intestinal flora, and regulating the expression of genes related to fat metabolism.

To further enhance GLP-1 stability, specific amino acid modifications have been made. The Ala at position 8 and Lys at positions 26 and 34 in the DNA sequence of mature active human GLP-1 (7–36) are sensitive to DPP-4 and trypsin. The alteration of these amino acids renders these enzymes incapable of recognizing GLP-1 effectively, thereby increasing half-life of GLP-1.[124] For example, Wang et al[125] mutated Ala8 to Gly8 in human GLP-1(7–36) to prevent DPP-4 recognition, and Lys26 and 34 to Gln26 and Asp34 to inhibit trypsin digestion. These modified GLP-1 genes, which are suitable for expression in *L. plantarum* WCFS1, were cloned into the plasmid pSIP403. Recombinant *L. plantarum* WCFS1 demonstrated improved glucose control, including lower fasting blood glucose levels, extended blood glucose maintenance, inhibited glucagon secretion, promoted β-cell proliferation, and reduced renal damage.

In addition, researchers have explored the use of tandem repeats of GLP-1 to enhance its stability and resistance to enzymatic degradation. Hou et al[126] constructed plasmids with multiple GLP-1 repeats (2×, 4×, and 8× GLP-1) via sequential ligation and transformation in *E. coli* BL21(DE3) cells. The resulting strains successfully secreted GLP-1 analogs, which reduced serum glucose levels in diabetic rats. Subsequently, 10×GLP-1 was successfully generated by a similar method.[127] It is worth mentioning that Fangfang Xu et al[128] further improved stability by adding cysteine residues to form a disulfide bond between GLP-1 dimers. This approach has been successfully applied to probiotics and has demonstrated enhanced stability and efficacy.[129]

Enhancing the expression and efficiency of GLP-1 in probiotics can improve their therapeutic outcomes. Wu et al[130] constructed an efficient expression vector, pNK1-PGK-10×GLP-1, and transformed it into *Saccharomyces cerevisiae* INVSc1. The recombinant yeast, named lLHY168, expressed GLP-1 at 1.56 mg/g cell wet weight with stable gene retention. The engineered yeast showed significant benefits in treatingT2DM mice.

## GLP-1RAs

GLP-1RAs represent a novel class of glucose-control drugs that have gained widespread use in the treatment of T2MD in recent years. These agonists mimic GLP-1 by binding to its receptor and promoting insulin secretion, thus aiding in the regulation of blood sugar levels. One key advantage of GLP-1RAs is their structural homology with GLP-1, which makes them resistant to degradation by DPP-4, thereby extending their half-life and enhancing their therapeutic potential.[131]

Exendin-4 (Exd4) is a crucial component of GLP-1RAs. It is a 39-amino-acid peptide with a 53% sequence homology to human GLP-1.[132] Exenatide, the first GLP-1RA drug, was approved by the US FDA in 2005 as an adjunctive therapy to improve blood sugar control in T2DM patients.[133] Zeng et al[134] demonstrated the feasibility of introducing Exd4 into probiotics, enabling engineered probiotics to produce and deliver Exd4 directly to the gut. They optimized the codon sequence of Exd4 for lactic acid bacteria and inserted it into the plasmid vector pMG76e, which also included the signal peptides Usp45 and LEISSTCDA, *Exd4*, a 6×His tag, and restriction sites XbaI and XhoI. The transformation of *L. paracasei* L14 with this recombinant plasmid resulted in the successful production of active Exe-4, which was able to cross the Caco-2 cell monolayer, promote insulin secretion, enhance β-cell proliferation, and reduce apoptosis. Subsequently, the same research group[135] refined their methodology by synthesizing an oligonucleotide that included the propeptide sequences of Usp45 and LEISSTCDA, codon-optimized *Exe-4*, and restriction sites SacI and Hin dIII. This oligonucleotide was inserted into the NICE vector pNZ8048, which was then used to transform *L. lactis* NZ9000. The maximum concentration of Exd4 secreted by recombinant Lactococcus lactis was 249 nmol/L at a bacterial concentration of $6\times10^8$ cfu/mL. This secretion significantly stimulated insulin production in rat pancreatic β cell line INS-1, reaching 69.96±0.74 ng/mL, which outperformed the previous preparation methods.

## Antigens and ILs

T1DM is an autoimmune disease in which insulin or other hypoglycemic drugs can alleviate symptoms, but do not address the underlying cause. Currently, antigenic vaccines are promising immunotherapies for treating T1DM.[136] Robert et al[99] have highlighted the potential of recombinant *L. lactis* as an effective tool for inducing antigen-specific oral tolerance. Liang et al[137] successfully expressed a fusion protein (Hsp65-6×P277) in *E. coli* BL21 (DE3) using the plasmid pET28-Hsp65-6×P277. This protein elicits an anti-inflammatory immune response, thus providing a potential vaccine against T1DM. However, the instability of HSP65 requires further refinement. Ma et al[138] constructed pHJ: HSP65-6P277 by replacing the nisin-inducible promoter PnisA with the constitutive promoter P32, thereby enhancing stability and expression in *L. lactis* NZ9000. This modified strain showed lower T1DM incidence and reduced islet inflammation in treated mice than in controls.

Furthermore, Kun-Feng Liu et al[139] took a different approach, integrating the PCR product 6IA2P2 with plasmids pCYT: HSP65-P277 and pHJ: HSP65-6P277 to generate pCYT: HSP65-6IA2P2 and pHJ: HSP65-6IA2P2. Transformed *L. lactis* NZ9000 exhibited prolonged expression in the intestinal mucosa and long-lasting immunoregulatory effects, effectively regulating blood sugar levels and reducing insulitis in NOD mice.

Another promising antigen is the colonization factor antigen I (CFA/I), which has shown efficacy in evoking bystander immunity and accelerating the development of antigen-specific Tregs. Massimo Maddaloni et al[140] synthesized pBzMM153 containing the *cfaI* operon, which was transformed into *lactis* IL1403. The recombinant strain regulates the immune system, ameliorates intestinal flora, and provides long-term protective effects.[141,142]

ILs play an important role in the immune response. The strategy of expressing ILs genes in probiotics has shown the potential for enhancing antigen-induced immune responses. *L. lactis* expressing IL-10 has been established to alleviate murine colitis,[143] a technique adapted for DM treatment.[144] The main strategy is to insert ILs gene in the developed expression system in lactic acid bacteria, which make the lactic acid bacteria has the ability of expressing and secreting ILs.[145] Recombinant *L. lactis* expressing both proinsulin and IL-10 have been successfully used to prevent T1DM.[146–148] For instance, Robert et al[149] further demonstrated the use of recombinant *L. lactis* expressing glutamic acid decarboxylase 65 and IL-10 to effectively prevent T1DM by improving immune regulation and reducing inflammation.

### Other Proteins

Genetic engineering has enabled the introduction of various proteins into probiotics for the treatment of DM. These engineered probiotics can produce specific proteins with therapeutic effects on DM. In addition to the categories mentioned in the previous section, notable examples of this approach exist.

Staphylococcal Nucleases: Chinese researchers developed a strain of *L. lactis* that expresses staphylococcal nucleases (CN Patent 201610353343.5), targeting DM treatment.[150] This genetically modified probiotic can disrupt neutrophil extracellular traps, reduce inflammation, regulate blood glucose levels, and delay the onset of diabetes with lower mortality and morbidity.

L-Arabinose Isomerase: Moez Rhimi et al[151] engineered *L. lactis* to express L-arabinose isomerase, which can convert galactose into tagatose, a sugar with a low glycemic index, thereby reducing hyperglycemia. This modification utilized an expression system developed for *L. lactis*, enhancing its potential for diabetes management.

## Challenges and Innovations in Clinical Translation of Engineered Probiotics

### Clinical Innovative Applications and Prospects of Engineered Probiotics

Engineered probiotics have garnered attention for their multifaceted roles in managing diabetes and associated complications. This section discusses their distinct advantages, synergistic effects, and their broader applications across different diseases, particularly in addressing diabetic complications.

#### Advantages of Engineered Probiotics in Diabetes Treatment

Engineered probiotics offer several unique advantages that distinguish them from traditional treatments for diabetes. These advantages include:

1. Targeted metabolic modulation: Engineered probiotics can produce bioactive compounds such as GLP-1, which directly enhances insulin secretion and improves glucose metabolism.[123] This allows for a more precise regulation of blood glucose levels.
2. Restoration of gut microbiota: Engineered probiotics contribute to a healthier gut microbiota, which is vital for enhancing insulin sensitivity and reducing systemic inflammation.[152] Balanced gut flora is increasingly recognized as a crucial factor in managing diabetes and preventing complications.
3. Non-invasive and patient-friendly: Probiotics, available in oral formulations, offer a non-invasive alternative to insulin injections, improving patient adherence.[153]
4. Personalized treatment potential: Engineering probiotics allows for customization based on an individual's microbiome profile, facilitating personalized therapeutic interventions that are tailored to the specific needs of each patient.[154]

#### Multiple Probiotics or Synergies Between Probiotics and Drugs

While single-strain probiotics are beneficial, multi-strain probiotics offer enhanced biological activity due to the additive and synergistic effects among individual strains. Research has shown that multi-strain probiotics can significantly improve glucose metabolism, modulate immune responses, and restore gut microbiota more effectively than single-strain formulations.[155]

Besides, the co-administration of probiotics with herbal therapies demonstrates significant synergistic effects. Recent studies have shown that combining probiotics with specific herbal extracts (such as garlic, fenugreek, and berberine) enhances therapeutic outcomes. These herbs, when co-administered with probiotics, optimize glucose metabolism, reduce insulin resistance, and modulate gut microbiota.[156,157] Similarly, when engineered probiotics are co-administered with active pharmaceutical ingredients (APIs) like metformin, they can improve drug bioavailability, enhance gut health, and mitigate gastrointestinal side effects, such as those commonly associated with API treatments.[158]

#### Engineered Probiotics and Diabetic Complications

Engineered probiotics play a crucial role in mitigating diabetic complications, which are often driven by chronic inflammation, oxidative stress, and endothelial dysfunction. Diabetic complications, such as neuropathy, retinopathy,

and cardiovascular diseases, can be alleviated through the use of probiotics that modulate gut microbiota, reduce systemic inflammation, and improve lipid profiles.[159,160]

Furthermore, while our focus remains on diabetes, the potential applications of engineered probiotics extend to other conditions, such as inflammatory bowel disease and metabolic syndrome, or even Alzheimer's disease.[143,161–163] Their ability to restore gut microbiota balance and enhance immune responses makes them valuable candidates for a variety of therapeutic contexts.

## Clinical Translation Challenge of Engineered Probiotics

While engineered probiotics hold great promise for diabetes management, several challenges must be addressed for successful clinical application.[164] These include ensuring the stability and viability of probiotics during storage and delivery, navigating complex regulatory landscapes, and generating robust clinical trial data to validate efficacy and safety. Additionally, understanding patient-specific factors, such as individual microbiome profiles, is crucial for optimizing therapeutic outcomes (Figure 6). Addressing these issues will be essential for translating engineered probiotics from research into routine clinical practice.

### Safety Concerns

Most engineered probiotics are genetically modified organisms (GMOs). These probiotics express the desired substances by accepting foreign recombinant plasmids, which are under strict regulatory scrutiny.[165] Such GMOs are prone to mutations or plasmid loss, leading to the loss of therapeutic substance expression in subsequent generations and the potential production of harmful substances, posing risks of genetic instability. Furthermore, the inclusion of antibiotic-

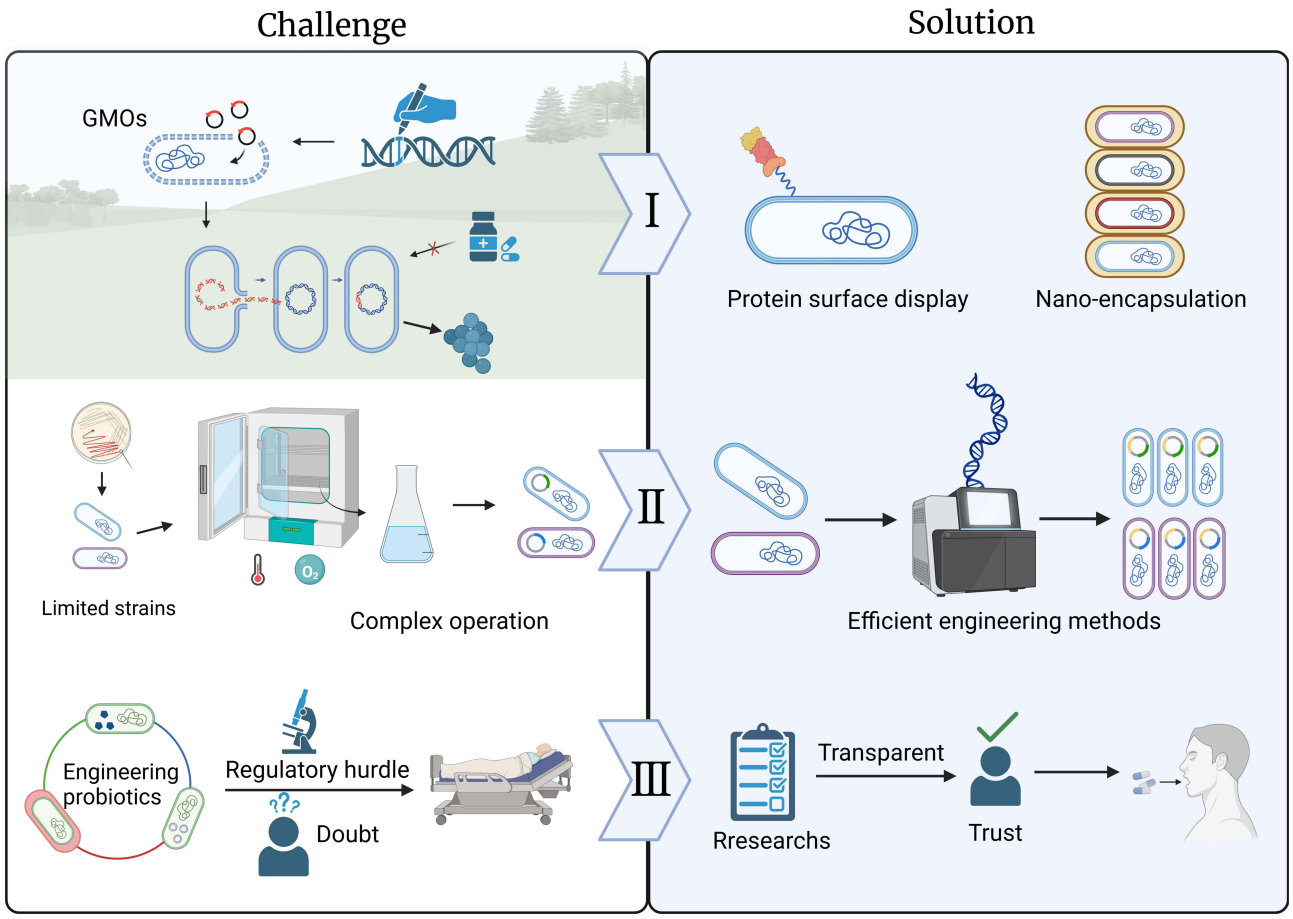


**Figure 6** The challenges and corresponding innovation in clinical translation of engineered probiotics.

resistance genes in plasmids for screening during production poses additional safety concerns. The release of probiotics with these resistance genes into the environment can lead to the transfer of resistance genes to other microorganisms, thereby exacerbating the antibiotic resistance crisis. Not all orally administered probiotics can colonize the gut, and some are excreted and proliferate in nature, potentially contaminating the environment.

To address these issues, it is crucial to develop more efficient and user-friendly genetic engineering methods to reduce operational complexity and costs, while minimizing potential risks. Innovation of recombinant protein surface display technologies to link therapeutic proteins to the surfaces of unmodified probiotics can help avoid the use of GMOs and ensure drug targeting and efficacy. Moreover, accelerating the transition from traditional microencapsulation to nanoencapsulation technologies can enhance the survival rate of probiotics and enable their large-scale application.

### Operational Challenges

The probiotics used in the gut are predominantly anaerobes, which are highly sensitive to oxygen, posing significant challenges in their isolation, cultivation, industrial production, and formulation. Genetic engineering techniques require high operational standards and many microorganisms lack manipulable genetic tools. Current research focuses on a limited subset of the vast microbial family, restricting modifications to a few strains.[31] Moreover, the stringent safety requirements for engineered probiotics have increased technical demands, such as prohibiting the use of antibiotic resistance genes in probiotics, complicating the screening process, or designing probiotics that do not proliferate extensively in the environment, necessitating the creation of nutritionally deficient strains.

Enhancing scientific and technological advancements to develop more efficient genetic engineering methods can reduce operational complexity and costs, while ensuring the safety of production strains. Exploring and developing surface display technologies and nano-encapsulation methods can enhance probiotic viability and facilitate large-scale probiotic modifications and application.[100]

### Regulatory and Public Acceptance

Regulatory hurdles and public skepticism towards GMOs significantly impede the clinical translation of engineered probiotics. Strict regulations govern the use of GMOs owing to their potential environmental and health risks. Additionally, public acceptance of GMOs remains low, further complicating their clinical adoption.

Extensive research and public outreach to demonstrate the safety and efficacy of engineered probiotics, engaging with regulatory bodies to streamline the approval processes for probiotics with well-documented safety profiles and promoting transparency in the development and testing of engineered probiotics can build public trust and acceptance.

## Conclusion

The intricate relationship between the gut microbiota and diabetes highlights the potential of engineered probiotics to manage the disease. Although conventional probiotics have demonstrated efficacy, their limitations necessitate advancements in engineering techniques. This review details the physical, bioenrichment, and genetic modifications that enhance the probiotic stability, functionality, and therapeutic efficacy. Future studies should focus on elucidating the mechanisms of action, improving the genetic engineering methods, and developing novel therapeutic proteins. Engineered probiotics offer a promising alternative to conventional therapies, potentially reducing side effects and expanding treatment options for diabetes and other diseases. The successful application of these innovations could mark a significant milestone in diabetes management and in broader medical applications.

## Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

## Funding

This work was supported by the National Natural Science Foundation of China (51901160), Natural Science Foundation of Zhejiang Province (LY22H020005), and Graduate Scientific Research Foundation of Wenzhou University (3162024003057, 3162024003056, and 3162024004102).

## Disclosure

The authors report no conflicts of interest in this work.

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