Food Chemistry: X 13 (2022) 100196

Contents lists available at ScienceDirect

Food Chemistry: X

journal homepage: www.sciencedirect.com/journal/food-chemistry-x

Bioactive peptides produced by engineered probiotics and other food-grade bacteria: A review

Haydee Eliza Romero-Luna [a], Adrián Hernández-Mendoza [b], Aarón Fernando González-Córdova [b], Audry Peredo-Lovillo [a,*]

[a] Subdirección de Posgrado e Investigación, Instituto Tecnológico Superior de Xalapa, Xalapa 91096, Veracruz, Mexico
[b] Laboratorio de Química y Biotecnología de Productos Lácteos, Centro de Investigación en Alimentación y Desarrollo A.C. (CIAD, A.C.), Hermosillo 83304, Sonora, Mexico

ARTICLE INFO

Keywords:
Synthetic biology
Genetic engineering
Gut microbiota
Lactic acid bacteria
Proteolytic system

ABSTRACT

Synthetic biology is employed for the study and design of engineered microbes with new and improved therapeutic functions. The main advantage of synthetic biology is the selective genetic manipulation of living organisms with desirable beneficial effects such as probiotics. Engineering technologies have contributed to the edition of metabolic processes involved in the mechanisms of action of probiotics, such as the generation of bioactive peptides. Hence, current information related to bioactive peptides, produced by different engineering probiotics, with antimicrobial, antiviral, antidiabetic, and antihypertensive activities, as well as their potential use as functional ingredients, is discussed here. Besides, the effectiveness and safety aspects of these bioactive peptides were also described.

Introduction

The human body is colonized by diverse microbial communities, collectively known as microbiota, which is attached to epithelial surfaces such as skin, urogenital tract, and gastrointestinal tract – GIT (Chua, Kwok, Aggarwal, Sun, & Chang 2017; Kohl, Castillo, & Ochoa-Repáraz, 2020; Zhou et al., 2020). The GIT microbiota contributes to the host health by helping in the proper food digestion, supplying bacterial metabolites with beneficial effects (e.g., SCFA, organic acids), breaking down toxins and stimulating the human immune system (Sola-Oladokun, Culligan, & Sleator, 2017; Zhou et al., 2020; Beltrán-Barrientos, Garcia, Hernández-Mendoza, González-Córdova, & Vallejo-Cordoba, 2021). Hence, considering that GIT microbiota composition is intimately connected with host health, its manipulation (either using prebiotics or probiotics) has emerged as a novel therapeutic target toward a broad spectrum of diseases (Kumar et al., 2016; Kohl et al., 2020).

Particularly, administration of conventional probiotics has been suggested as a strategy to restore microbial dysbiosis and maintain the microbial balance by preventing colonization of pathogenic bacteria either by competing for nutrients and adhering to epithelial/mucus surfaces, antagonizing pathogen colonization through aggregation with pathogens, or by modulating the immune system (Chugh & Kamal-Eldin, 2020; Zhou et al., 2020).

Despite this, some conventional probiotic strains have shown certain limitations, for instance, probiotic potential can differ in certain hosts, they may harbor transmissible antibiotic resistance determinants or produce undesirable metabolites, such D-lactate, ammonia, biogenic amines, among others (Kothari, Patel & Kim, 2019; Sotoudegan et al., 2019). Additionally, conventional probiotics may be nonspecific for different pathogens, as the antimicrobial substances they release are limited to specific microbes (Mathipa & Thantsha, 2017; Zuo, Chen, & Marcotte, 2020). Therefore, genetic engineering techniques have been exploited to design probiotics with improved beneficial features (Yadav & Shukla, 2019). Potential applications of such engineered probiotics include their use as delivery agents of drugs and vaccines as well as cell factories to produce desired metabolites such as bioactive peptides (Sola-Oladokun et al., 2017; Mays & Nair, 2018; Yadav, Kumar, Baweja, & Shukla, 2018; Veiga, Suez, Derrien, & Elinav, 2020; Mejía-Pitta et al., 2021).

In this last regard, bioactive peptides from engineered probiotics have shown a broad pattern of biofunctions including, but not limited to, antimicrobial, antiviral, antidiabetic, and antihypertensive activities

* Corresponding author.
E-mail addresses: roluna88@hotmail.com (H.E. Romero-Luna), ahernandez@ciad.mx (A. Hernández-Mendoza), aaronglz@ciad.mx (A.F. González-Córdova), aperedo88@outlook.com (A. Peredo-Lovillo).

https://doi.org/10.1016/j.fochx.2021.100196
Received 20 August 2021; Received in revised form 9 November 2021; Accepted 22 December 2021
Available online 22 December 2021

(Sánchez & Vázquez, 2017; Chakrabarti et al., 2018; Chen et al., 2021). Hence, the present review aimed to provide an overview of the biological activities, effectiveness, and safety aspects of bioactive peptides produced by engineering probiotics, as well as their potential use as functional ingredients.

Conventional probiotics

Generalities

Conventional probiotics are commonly Gram-positive lactic acid bacteria (LAB), mainly belonging to the genera *Lactobacillus* and *Bifidobacterium*. However, other non-LAB have also been considered, including *Bacillus coagulans* GBI-30, *Escherichia coli* Nissle 1917, and *Saccharomyces cerevisiae* subsp. *boulardii* (Ayichew et al., 2017; Mays & Nair, 2018).

Is fairly well known that when conventional probiotics are frequently consumed in adequate amounts, they can influence the host's nutrition, metabolism, physiology, and defenses (including innate and acquired), which confer different health benefits to human in a strain-dependent manner, including immune system modulation and increased resistance to infectious illness (Ayichew et al., 2017; Yadav et al., 2018; Aggarwal et al., 2020). Although the general effectiveness of traditional probiotics has been associated with their ability to survive the hostile gut environment, as well as to adhere and colonize the GIT, the mechanism of action by which exert their biological effects are varied and cannot be generalized to all probiotics (Evivie, Huo, Igene, & Bian, 2017). It should be highlighted that some health effects of probiotics and their modes of action have been well-documented but are beyond the scope of this review and have been exhaustively discussed elsewhere (Plaza-Diaz, Ruiz-Ojeda, Gil-Campos & Gil, 2019).

On the other hand, despite the number of health benefits endorsed to probiotics, it should also be pointed out that probiosis may differ in certain people due to host factors (e.g., personalized gut mucosal colonization resistance to some probiotics), co-morbidities, and certain medical conditions (Zmora et al., 2018; Kothari et al., 2019); which poses potential downsides to probiotics, as described below.

Potential drawbacks of conventional probiotics

Loss of viability during downstream processing operations, sensitivity to environmental changes, and limited knowledge of the mechanism of action within GIT conditions are factors that limit the functionality of conventional probiotics and make it difficult to optimize their beneficial effects (Nguyen, Truong, Kouhoundé, Ly, Razafindralambo, & Delvigne, 2016; Bober, Beisel, & Nair, 2018; Mazhar et al., 2020; Zhou et al., 2020).

Furthermore, evidence has shown that some people may experience side effects related to probiotic ingestion. Such effects tend to be mild and digestive problems (such as gas or bloating); however, more serious outcomes have been reported in certain at-risk populations, especially in individuals receiving antibiotic treatment or those severely immune compromised (Shenderov et al., 2021).

Thus, in order to minimize these potential limitations, new probiotic strains have been designed to obtain improve interactions with the host cells, as well as to induce defined mechanisms of action and production of specific microbial metabolites (Yadav & Shukla, 2019). According to this, the employment of omics technologies has emerged as a useful tool to produce a new generation of engineered probiotics with novel therapeutic functions, including the production and delivery of therapeutic metabolic products (Zuo et al., 2020).

Engineered probiotics

Definition and characterization of engineered probiotics

Synthetic biology is a growing field used to design and achieve programmed probiotic behavior using natural or synthetic biological components, based on the design-build-test-learn cycle (Son & Jeong, 2020). This type of engineering has opened a wide range of opportunities by using bacteria to stimulate the host's immune system, to improve microbial metabolic systems to produce desirable compounds, to combat pathogens, and to design genetic circuits for diseases detection, as shown in Fig. 1 (Bober et al., 2018; Senapati, Dash, Sethi, & Chakraborty, 2020; Zuo & Chen, 2020). For instance, modified probiotics can deliver antitumor agents *in situ* and prevent damage to healthy cells. Thus, the designed probiotics obtained through genetic transformation may change the conventional therapeutic treatments used in disease management (Hag & Poondla, 2021).

Engineered probiotics can be defined as microorganisms with optimized metabolic processes achieved by the applications of omics technologies focused on increasing their probiotic potential (Aggarwal et al., 2020; Mazhar et al., 2020). In this sense, metabolic engineering is a multidisciplinary field in which the production of desirable fermentation products and metabolites is induced or improved in the probiotic cells (Yadav et al., 2018). Lactococci and Lactobacilli are the most widely LAB used for the development of engineered bacteria (Kohl et al., 2020). Although *Lactococcus* strains are unable to colonize the human GIT (Kohl et al., 2020), and have not been recognized as probiotics, they have potential, since they have previously shown GRAS status, anti-inflammatory, immunomodulatory, antimicrobial, and technological properties (Liu et al., 2019; Jawan, Abbasiliasi, Mustafa, Kapri, Halim, & Ariff, 2020; Delgado-Venegas et al., 2021); hence, a couple of examples of engineered bacteria will be illustrated below with specific *Lactococcus* strains.

Genetic design tools used to engineer probiotics and other food-grade LAB

Engineering techniques include synthetic biology approaches for the genetic designing of probiotics, including two primary strategies, i) top-down approach (genome reduction to delete the non-essential genes), and ii) bottom-up approach (genome synthesis to add essential genes parts). Through either of these two strategies, the biochemical pathways of probiotics can be altered to promote, enhance, or terminate specific bioactivities, based on efficient and precise genome editing tools (Yadav et al., 2018; Son & Jeong, 2020; Zuo & Chen, 2020).

Incorporation of foreign DNA is a genome-editing tool by which plasmid DNA, in the form of single-stranded, is transferred into conventional probiotics by conjugation, transformation through chemical, or electric disruption of the cell membrane, induction of natural competence or by phage transduction (Bron et al., 2019; Zuo et al., 2020). Similarly, gene expression is another engineering technique that introduces foreign DNA to bacterial cells to improve the expression of genes related to specific metabolic pathways. However, to obtain an efficient expression and correct localization of the recombinant gene, promoter and regulatory elements, ribosomal binding site and localization signals (secretion signals and cell surface anchoring elements) must be considered (Zuo et al., 2020). These engineering techniques have been used to activate desirable traits in probiotics such as *Lactobacillus casei* BL23, *Lb. plantarum* WCFS1, and *Escherichia coli* Nissle 1917; particularly to confer tolerance to specific bile salts, and to produce and secrete antimicrobial peptides to specifically target and kill *Enterococcus* spp., respectively (Geldart et al., 2015; Martínez-Fernández, Bravo, Peirotén, Arqués, & Landete, 2019).

Another genomic engineering tool is based on the use of temperature-sensitive plasmid systems (TSPS), specifically for the genome engineering of Bifidobacteria. One of these TSPS is composed by the pORI19/pTGB019 two plasmid system, in which the thermosensitive

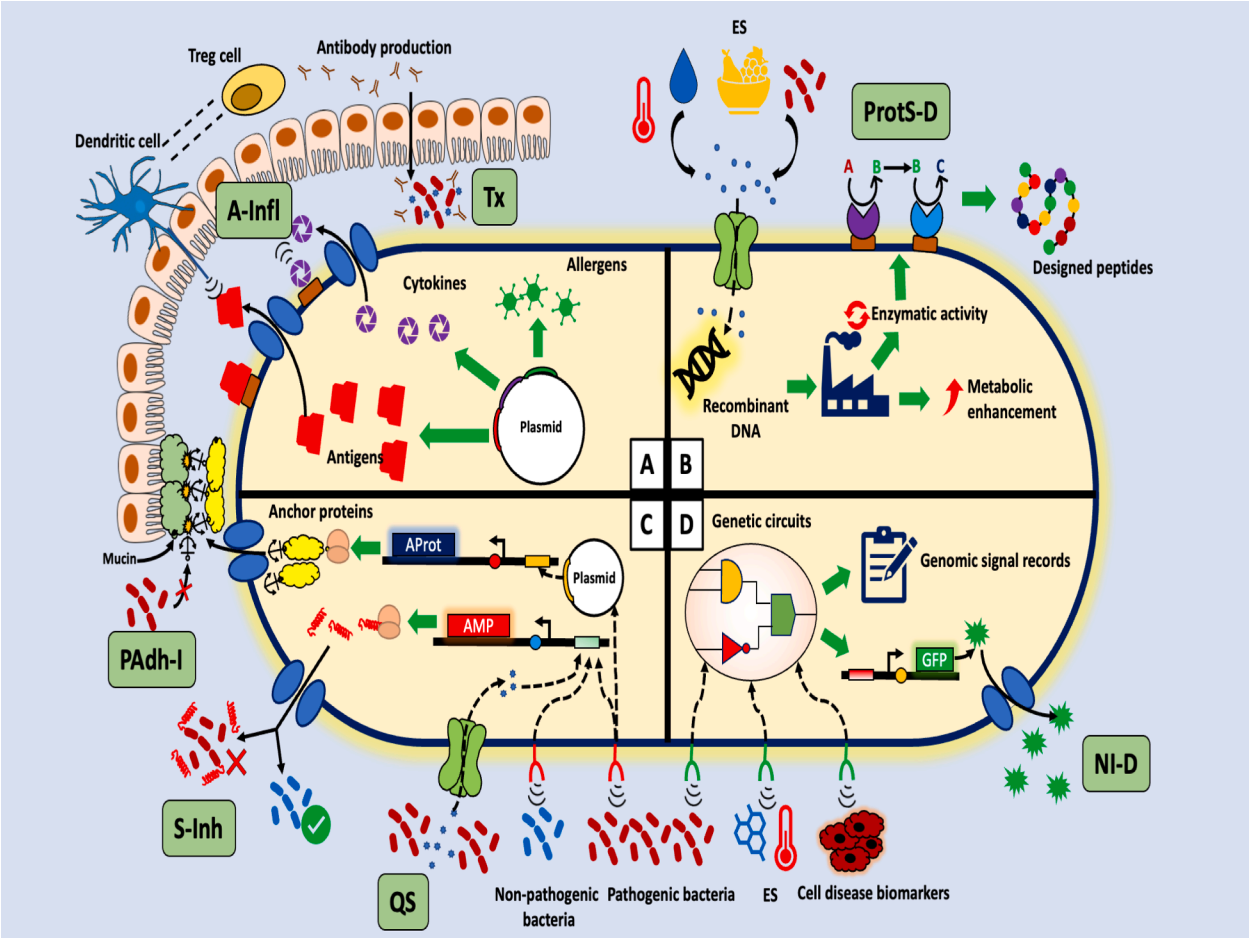

Fig. 1. Therapeutic approaches of engineered probiotics. Different synthetic biology tools are applied in probiotic bacteria to induce an immune response from the host cells (A), improve microbial metabolic systems (B), combat pathogens (C), and design genetic circuits for sensing and diagnosing diseases (D). Engineered probiotics interact with the mucosal immune system and can deliver synthetic antigens, cytokines, and allergens to generate anti-inflammatory effects (A-Infl) and inhibition of toxigenic microbes (Tx-Inh) through the generation of host antibodies. Probiotics can be engineered through recombinant DNA insertion to increase their metabolic pathways and design their proteolytic systems (ProtS-D) to obtain predicted bioactive metabolites, as a response to specific environmental stimuli (ES) (temperature, water, diet, and microbes). Antimicrobial effects in engineered probiotics are triggered by microbial stimuli from pathogenic and non-pathogenic microbes, and by chemical signals from quorum sensing (QS) between specific bacteria. These stimuli induce the expression of genes for the synthesis of antimicrobial peptides (AMP's) and anchor proteins (AProt) to perform selectively inhibition (S-Inh) of bacteria and prevention of pathogens adhesion to epithelial mucin, respectively. Genetic circuits, used as non-invasive diagnostics (NI-D), can detect ES, pathogenic bacteria, and cell disease biomarkers, to diagnose disease through a reporter green fluorescent protein (GFP). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

pTGB019 plasmid facilitates the replication of the non-replicable pORI19 plasmid and the liberation of DNA homologs. Additionally, the pKO403 is a single plasmid system applied to generate gene deletion in engineered *Bifidobacterium longum* 105-A and *B. longum* NCC2705 (Sakaguchi, Funaoka, Tani, Kano, & Suzuki, 2012).

Similarly, the genome editing via clustered regularly interspaced short palindromic repeat/CRISPR-associated protein (CRISPR-Cas) system promotes a rapid and efficient genome modification that have been extensively used to create the tailored systems engineering of LAB and *Bifidobacterium* probiotic strains (Hidalgo-Cantabrana, O'Flaherty, & Barragou, 2017; Zuo et al., 2020). CRISPR-Cas is a genetic engineering tool for eukaryotic cells; however, this system is also widespread in LAB (including *Lactobacillus*, *Lactococcus,* and *Streptococcus* species), Bifidobacteria and other members of the human microbiome, due to the frequent exposition of these bacteria to phage or foreign DNA present in dairy environments, fermented foods, or GIT (Hidalgo-Cantabrana et al., 2017). The CRISPR technique has been used to design engineered probiotics with health functionalities using conventional LAB probiotics as chassis and modifying their inherent functions to provide stability based on their own CRISPR-Cas systems (Hag & Poondla, 2021).

As seen in Fig. 1D, the cell behavior can be reprogrammed by initiating gene expression as a response to specific signals from genetic regulatory circuits (Zuo et al., 2020). Bacterial genetic circuits can respond to molecules that are relevant for health or disease, including cytokines and hormones, as well as physiological stimuli from GIT such as temperature and metabolites produced either during epithelial infection or inflammation (Riglar & Silver, 2018). Therefore, the controlled expression of genes based on the ability of LAB and probiotics to recognize signals from GIT (e.g., pH, bile, inflammation markers, toxic metals, pathogenic microorganisms, or microbial compounds), can be used for the construction of genetic circuits that allow the engineering of microorganisms with therapeutic and diagnostic purposes, either as recognizers of physiological alterations, or as producers of bioactive metabolites with a controlled and specific release (Bradley, Buck, & Wang, 2016; Braff, Shis, & Collins, 2016; Xia, Ling, Foo, & Chang, 2019; Zuo et al., 2020).

In this context, engineered probiotics can be used as living therapeutic agents for the mucosal delivery of prophylactic and therapeutic enzymes, DNA, cytokines, allergens, and bioactive peptides, with many advantages such as stability, the lower delivery cost of substances at the mucosal surface, and increased shelf life (Mazhar et al., 2020). Indeed, bioactive peptides from engineered probiotics have gained increasing

recognition in the last years due to their multiple functionalities. These bioactive peptides are released by the engineered proteolytic system of designed probiotics with different pre-controlled activities, that could be adequate for a desired physiological response (Piñero-Lambea, Ruano-Gallego & Fernández, 2015). Hence, to obtain bioactive peptides with specific functionalities, the proteolytic system of designed probiotics should be shifted using one or a combination of the synthetic genetic design tools previously described.

Edition of proteolytic systems

Probiotics can hydrolyze proteins from the external environment through their proteolytic systems, which consist of the cell-envelope proteinases (CEPs), dipeptide (Dpp), di/tripeptide (DtpT) and oligopeptide (Poo) transport systems, and intracellular peptidases. Once internalized, the peptides are degraded into amino acids by numerous internal peptidases such as endopeptidases, aminopeptidases, tripeptidases, dipeptidases, and proline-specific peptidases (Raveschot et al., 2018).

Proteolytic activities vary between species and strain specificity in protease production has been observed within bacteria from same species, probably due to the differences in CEPs gene expression, CEPs gene mutations and differences in optimal conditions for enzymatic activity (Raveschot et al., 2018). Therefore, it is important to understand the regulatory mechanisms involved in the synthesis of microbial bioactive peptides, to later improve them through comparative genomic analysis and genomic engineering techniques. These genomic editing techniques have served as models to develop strategies that increase the production yield of specific bioactive peptides (Hafeez, Cakir-Kiefer, Roux, Perrin, Miclo, & Dary-Mourot, 2014; Mejía-Pitta et al., 2021).

In this sense, food-grade LAB, with probiotic potential, including *L. lactis*, *Pediococcus* spp., and *Enterococcus faecalis,* have been metabolically engineered to increase the production of nisin, pediocin, and enterocin, respectively (Plavec & Berlec, 2020). Several gene expression systems have been developed for heterologous peptide expression in LAB, being the nisin-controlled gene expression (NICE) system one of the most widely used inducible systems, involved in the biosynthesis of nisin A, a bacteriocin produced by several *L. lactis* (Škrlec et al., 2018). NICE P_{nisA} promoter is induced by the NisA peptide (nisin) through the two-component systems NikS and NikR, achieving a dynamic range of expression (up to 1000-fold) by adding increasing the concentration of nisin (Kohl et al., 2020). The application of synthetic biology on probiotics is focused on the design of engineered strains for their subsequent administration within GIT, where their metabolic promoters, including NICE, are activated by gut signals triggering controlled or induced gene expression and translation into specific functional metabolites, such as bioactive peptides (Mazhar et al., 2020).

Applications of genetically engineered bacteria

Genomic engineering techniques offer the opportunity to design food-grade LAB, including probiotics, with considerable clinical and biotechnological potential in different fields such as medical, agriculture, food, and feed. In this last sense, it has been observed that engineered probiotics can show better resistance to biotic and abiotic stress, in comparison to traditional probiotics (Yadav et al., 2018).

The main application of engineered probiotics occurs in functional foods design. The use of engineered probiotics for industrial purposes has been observed in milk and dairy fermentation. Particularly, *Streptococcus thermophilus* strains have been bioengineered to express stress-ameliorating enzymes, as well as to enhance their proteolytic system, expressing heterologous proteases or peptidases, to minimize the manufacture time and the possibility of unwanted contamination. Besides, the use of engineered microbes in the dairy industry may impact the development of reduced-sugar food products, flavor substances, and texturizing agents. At the same time, may improve the production of bioactive peptides with enhanced bactericidal effects such as nisin, pediocin, and enterocin, bacteriocins with food applications as an effective preservative agent against *Listeria* and *Clostridium* spores (Markakiou, Gaspar, Johansen, Zeidan, & Neves, 2020; Plavec & Berlec, 2020).

Milk and dairy products are recognized as the second most important source of proteins in foods. Such proteins serve as a substrate for the proteolytic machinery of LAB and engineered probiotics, to generate bioactive peptides with a wide range of functionalities (Hafeez et al., 2014). The engineered probiotics also improve the production of specific bacterial enzymes with limited hydrolytic activity, which prevent further hydrolysis of the produced bioactive peptides, avoiding an uncontrolled degradation and the reduction or loss of peptides bioactivities (Daliri, Lee, & Oh, 2018; Chai, Voo, & Chen, 2020).

On the other hand, therapeutic uses of engineered probiotics are mainly based on the localized and controlled release of antimicrobial agents under specific conditions. Engineering food-grade bacteria, such as probiotics, allows the creation of alternatives for the treatment and prevention of infectious diseases, either by the release of antimicrobial compounds (e.g., bacteriocins) or by expressing heterologous antigens that stimulate the immune system to produce relevant antibodies for long-term prophylaxis (Braff et al., 2016). Besides synthesis of bioactive peptides with antimicrobial activity, based on trial-and-error approaches, the design of other peptides with different functionality, following new metabolic engineering methods, such as the four-step synthetic biology system, which encompasses the design-build-test-learn process (Fig. 2), have also been reported. This has led to the development of bioactive peptides with antiviral, antidiabetic, and antihypertensive properties, among others (Son & Jeong, 2020).

Bioactive peptides from genetically engineered probiotics and other food-grade LAB

Bioactive peptides are defined as specific protein fragments composed of 2 to 20 amino acids (<6,000 Da) monomers that are initially inactive within the sequence of the parent protein (Chai et al., 2020). These encrypted protein fragments can be liberated from their parent proteins through hydrolysis of proteolytic enzymes from animals, plants, and microorganisms, either by fermentation (proteolytic strains) or digestion (gastrointestinal enzymes). Once liberated, these peptides can exert specific beneficial effects on human health (Taniguchi et al., 2018; Ali et al., 2021).

It is well known that different food-grade LAB, including probiotics, are capable to produce bioactive peptides; however, new engineering technologies have emerged as alternatives to obtain bacteria with a higher production rate and low cost, as well as a low toxicity, high rate of host response, but maybe more importantly, with bioactive properties that promise to be more efficient and safer for the treatment and prevention of diseases, compared to common synthetic drugs (Daliri et al., 2018). The main bioactivities of peptides generated by engineered probiotics will be described in the next sections; while the mechanisms through which these designed probiotics exert antimicrobial, antiviral, antidiabetic, and antihypertensive activities are displayed in Fig. 2.

Antimicrobial peptides

The actual mode of bacterial killing by engineered probiotics remains in a poor level of understanding. However, it has been proposed that the antibacterial effect is related to the production and release of specific and predicted antimicrobial peptides (AMPs), or by releasing antigenic peptides that stimulate the host immune response (Fig. 2). These bioactivities can be used to improve efficacy in the treatment of enteric infections caused by antibiotic-resistant pathogenic bacteria (Plavec & Berlec, 2019; Mazhar et al., 2020; Hag & Poondla, 2021).

Probiotics can also be designed to sense pathogenic microorganisms and then exhibit antimicrobial properties through the production of

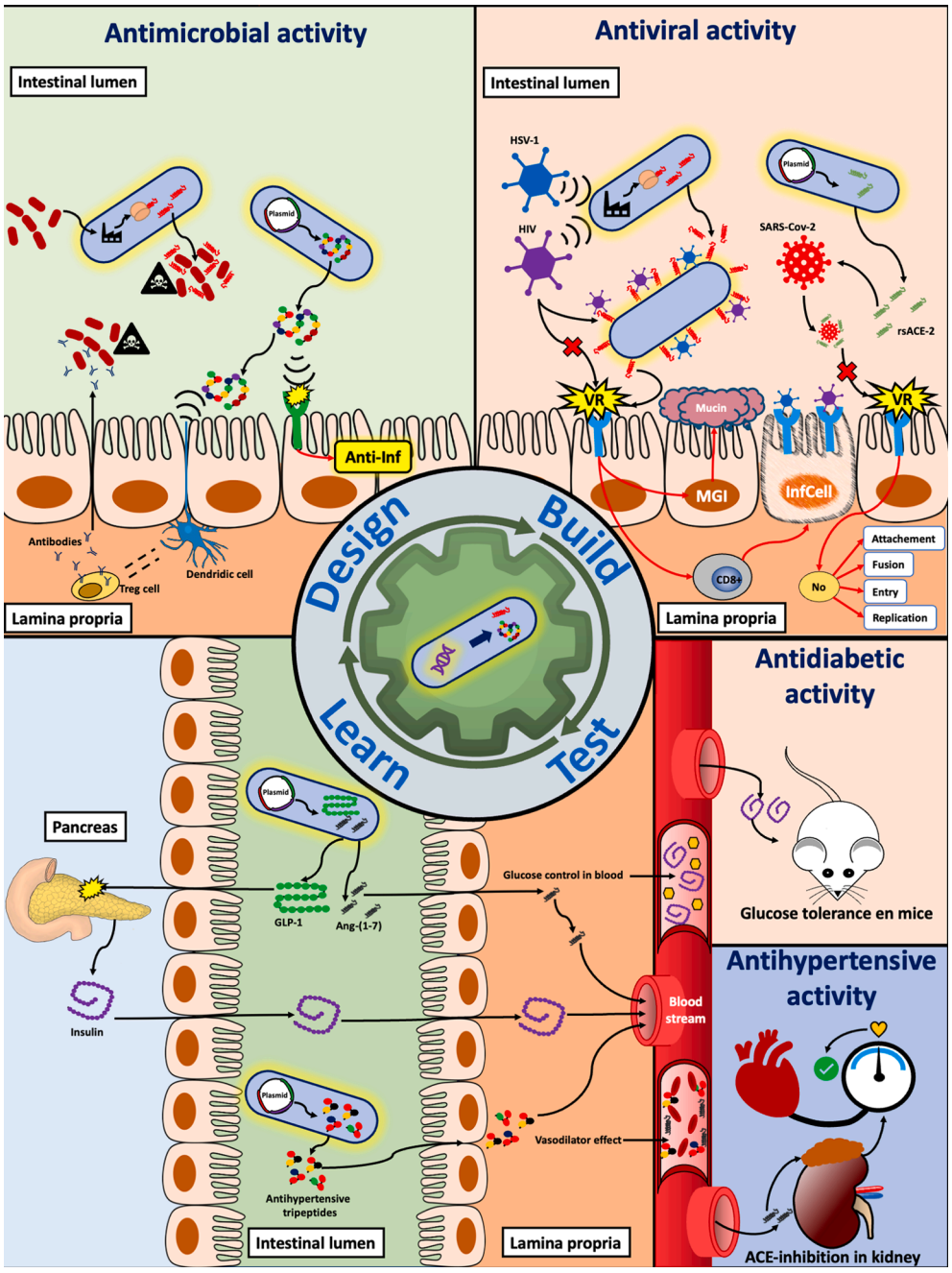

Fig. 2. Bioactivities of peptides produced by engineered probiotics. According to the four-step system of synthetic biology (Design-Build-Test-Learn), antimicrobial peptides eliminate pathogens by direct inhibition or by stimulation of host immune cells. Antiviral activity includes bacterial surface peptides that attach the virus before binding to epithelial receptors as well as induction of immune responses related to mucin generation and destruction of infected cells. Designed probiotics produce recombinant peptides capable of inactivating viruses such as SARS-CoV-2 preventing further viral infection. Also, antidiabetic and antihypertensive activities are based on the production of bioactive peptides useful to decrease glucose in the blood and with vasodilator properties to decrease blood pressure, in addition to acting as inhibitors of the hypertensive enzymatic system in the kidney. Anti-inf: Anti-inflammatory effect, BS: Binding site; SARS-CoV-2: severe acute respiratory syndrome coronavirus 2; rsACE-2: recombinant soluble angiotensin-converting enzyme 2; InfCell: infected cell, HSV-1: herpes simplex virus type 1; HIV: human immunodeficiency virus; GLP-1: glucagon-like peptide 1; Ang-(1–7): angiotensin-(1–7); ACE: angiotensin-converting enzyme.

both AMPs and bacteriocins (Bober et al., 2018). Bacteriocins with broader activity spectra are preferable for food preservations, while bacteriocins with narrower inhibitory spectra are more desirable under certain circumstances, since they may be effective against target bacterium but inactive against self-producer LAB. (Geldart et al., 2015). Renye and Somkuti, (2008) were the first to successfully engineer a LAB (*S. thermophilus*) to produce an antimicrobial peptide with a RRWQWRMKKLG sequence.

From that moment on, different authors have directed their studies in the use of engineered probiotics to produce and secret bacteriocins (Mandal et al., 2014; Borrero, Chen, Dunny, & Kaznessis, 2015; Braff et al., 2016). However, once bacteriocins reach the GIT, their activity is compromised mainly by intrinsic proteolytic degradation during digestion (Mejía-Pitta et al., 2021). To overcome this problem, engineered probiotics have been used as vehicles for bacteriocins delivering in clinical trials, since these bacterial are able to survive the GIT conditions (Braff et al., 2016).

For instance, Borrero et al. (2015) engineered a potential probiotic strain, *L. lactis* NZ9000, to limit the growth of *E. faecalis,* based on both recognition of the sex pheromone of *E. faecalis* and the consequent production of three bacteriocins (enterocin A, enterocin P and hiracin JM79). In related work, probiotic *S. boulardii* CNCM- I-745 was used to produce and deliver the antilisterial peptide leucocin C, by cloning the gene *lecC* from *Leuconostoc carnosum* into *S. boulardii* CNCM- I-745 (Li, Wan, Takala, & Saris, 2021). Similarly, other probiotic bacteria species have been used as cell factories as well as delivery vehicles of recombinant bioactive peptides with therapeutic application (Table 1).

On the other hand, the heterologous production of bacteriocins has been evaluated in *Lactobacillus* strains such as *Lb. casei* CECT475; *Lb. sakei* Lb790, and *Lb. plantarum* NC8. Cloned plasmids were inserted into bacteria to produce enterocin A. Engineered *Lb. casei* CECT475 showed a 4.9-fold higher production of enterocin A, with 15.7 to 59.2-fold higher

Table 1
Therapeutic uses of bioactive peptides from engineered probiotic and potentially probiotic strains.

Bioactivity	Peptide	Engineered probiotic and food-grade strains	Activity	Reference
Antimicrobial	Enterocin A, B and Hiracin JM79	*E. coli* Nissle 1917	Potent activity against *E. faecalis* and *E. faecium*	Geldart et al., 2018
	Dispersin B		Antimicrobial effect based on recognition of *Pseudomonas aeruginosa* and anti-biofilm activity in *Caenorhabditis elegans* and mice	Hwang et al., 2017
	Reuterin	*Lb. reuteri* ATCC PTA 6475	RecT gene insertion in *Lb. reuteri* to improve the synthesis of the bacteriocin reuterin to control infections	van Pijkeren et al., 2012
	Alysteserin, CRAMP[1] and Laterosporulin	*L. lactis* MG1363 (LMBP 3019)	Selective inhibition of *Helicobacter pylori* co-cultivated with *Lb. plantarum* and *E. coli* strains	Choudhury et al., 2021
	CRR6[2]	*Lb. gasseri* NM713	Significant protection after nasal challenge with *Streptococcus pyogenes*, indicating a potential use of recombinant *Lb. gasseri* as an oral vaccine against group A Streptococci	Mansour & Abdelaziz, 2016
Antiviral	HIV[3]-1 entry inhibitor cyanovirin-N	*Lb. jensenii* 1153–1666	Reduction in transmission of a chimeric simian/HIV[3] after repeated vaginal tests in macaques	Lagenaur et al., 2011
	Dendritic cell-targeting peptide fused with PEDV[4] core neutralizing epitope antigen	*Lb. casei* ATCC 393	Antiviral probiotic vaccine against PEDV[4] in pigs	Wang et al., 2017
Anti-inflammatory	Pentadecapeptide BPC-157	*L. lactis* NZ9000	Decrease ROS[5] concentration in fibroblast cells as possible treatment against IBD[6] and GIT[7] inflammations	Škrlec et al., 2018
	EDG[8], TFF[9]	*L. lactis* PSM565	Repair of mucosal epithelial barriers, accelerate cells migration	Huynh & Li, 2015
	Cathelicidin	*L. lactis* NZ3900	Reduction of inflammation in mice with colitis	Zhang et al., 2016b; Wong et al., 2017
	α-melanocyte-stimulating hormone	*B. longum* HB15	Anti-inflammatory effect during ulcerative colitis induction in rats	Wei et al., 2016
Anticancer	Kisspeptin	*L. lactis* NZ9000	Inhibition of HT-29 proliferation and migration, mediating apoptosis, down-regulation MMP-9[10] expression	Zhang et al., 2016a
	TFF[11]	*L. lactis* (sAGX0085)	Reduction of days with ulcerative oral mucositis in locally advanced head and neck cancer patients	Limaye et al., 2013
Immune stimulation	Myelin peptide fragments	*L. lactis* IBB360	Decrease of histopathological changes, reduction of serum IL-1b, IL-10 and TNF-α	Kasarello et al., 2016
	Gliadin	*L. lactis* MG1363	Suppression of local and systemic gluten-sensitive disorders in non-obese diabetic mice	Huibregtse et al., 2009

[1] CRAMP: cathelin-related antimicrobial peptide; [2]CRR6: conserved region of streptococcal M6 protein; [3]HIV: human immunodeficiency virus; [4]PEDV: porcine epidemic diarrhea virus; [5]ROS: reactive oxygen species; [6]IBD: irritable bowel diseases; [7]GIT: gastrointestinal tract; [8]EDG: epidermal growth factor; [9]TFF: trefoil factor; [10]MMP-9: matrix metalloproteinase.

antimicrobial activity against *Listeria* spp. than those from *Enterococcus faecium* T136, a natural bacteriocin producer (Jiménez et al., 2015). Additionally, Zhang et al. (2016b) evaluated the ability of engineered *L. lactis* NZ3900 strain to control *Helicobacter pylori* survival and infection, owing to its increased cathelicidin capacity production. Results showed that antimicrobial peptides inhibit pathogen growth, destroy the bacteria biofilm, and induce morphological alterations in its cell membrane.

According to the results described above, the use of engineered probiotics as an effective delivery system of AMPs in the gut, could avoid degradation, mitigate potential off-target effects, and significantly reduce AMPs production costs as self-replicating probiotics are less expensive than peptides synthesized by chemical reactions (Mejía-Pitta et al., 2021).

Antiviral peptides

The antiviral mechanism has been linked to the direct competition for the binding site on the host cell receptors. Thus, for the development of new antiviral probiotics, engineering techniques must be aimed to introduce recombinant surface peptides in order to increase their adhesion capacity to target receptors in the human body. Specifically, when probiotics bind to the epithelial surface, they may also induce the production of mucin, which interferes with the virus adhesion capacity. Then CD8 + T lymphocytes activation occurs, and the virus-infected cells are destroyed (Mandal, Pati, Chakraborty, & Franco, 2016). Through this mechanism, probiotics can stimulate an antiviral response from the GIT cells.

Additionally, engineered probiotics have been demonstrated to suppress the pathogenesis of harmful microbes at the enteric level by sequestering the microbes or their toxins. This binding capacity prompted the possibility to use engineered probiotics as removing agents for pathogenic microbes, including viruses such as human immunodeficiency virus (HIV) and severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). For instance, *E. coli* Nissle 1917 was engineered to colonize the human GIT and then secret hybrid peptides of the HIV-gp41-hemolysin A, throughout the luminal mucosa epithelial surface, which interfere with the HIV attachment, fusion, entry, or replication in target intestinal cells (Rao et al., 2005).

Recent studies have reported that GIT may act as an alternative route of infection for SARS-CoV-2, as some patients have manifested diarrhea as a related symptom (Liang et al., 2020). This could be associated with the presence of intestinal angiotensin-converting enzyme 2 (ACE-2), which has been identified as the receptor for the SARS-CoV-2 viral entry (Walls, Park, Tortorici, Wall, McGuire, & Veesler, 2020; Wang et al., 2020a,b). With this in mind, new antiviral strategies, including recombinant soluble ACE-2 (rsACE-2) have been developed, since rsACE-2 can block infection of SARS-CoV-2 at the GIT level. Besides, new strategies are focused on the use of engineered probiotics with expressing cells surface-bound or secretory rsACE-2, as a promising pharmacological tool to control the early stage of SARS-CoV-2 infection (Senapati et al., 2020).

Furthermore, considering that probiotics have shown antiviral potentials against several respiratory viral infections such as influenza and respiratory syncytial virus; and more recently, against coronavirus disease 2019 (COVID-19) (d'Ettorre et al., 2020; Moradi-kalbolandi, Majidzadeh-A, Abdolvahab, Jalili, & Farahmand, 2021); emerging trends in the ever-progressing field of vaccine development have emphasized the use, through nasogastric or orogastric routes, of engineered probiotics as promising vehicles for the delivery of non-invasive immunogenic molecules, which may protect against viral infections by improving humoral, mucosal and *T*-cell mediated immune responses (Taghinezhad-S et al., 2021).

These findings and the novelty relevance of the gut-lung axis for the

COVID-19 management, are the main factors that support the use of recombinant probiotics and their metabolites for the vaccine development of SARS-CoV-2. In this regard, recombinant *Lb. casei* Shirota was the first probiotic bacteria used to express coronavirus transmissible gastroenteritis virus spike (S) protein, as a live antiviral vaccine with a mucosal and systemic humoral response for one week after adhesion to jejunum and ileum (Moradi-kalbolandi et al., 2021). An example of this is the recombinant probiotic vaccine Symvivo Corportation (Biotech Company), which uses an engineered *Bifidobacterium longum* to produce the S protein after the expression of the gene *bacTRL-Spike*. This vaccine has shown effectiveness in the prevention of COVID-19 infection, potentiating the use of engineered probiotics not only as gastrointestinal protective tools but also as a new source of natural bioactive antiviral agents with no side effects (Wang et al., 2020b; Moradi-kalbolandi et al., 2021; Taghinezhad-S et al., 2021).

In addition to human applications, engineered probiotics are intended to produce antiviral peptides that have also been designed for poultry farming. For instance, vaccinated chickens with an antiviral peptide, constructed by expressing a conserved peptide from the ectodomain of M2 antigen in the surface of potentially probiotic *L. lactis* LM2301, showed a higher survival rate than those non-vaccinated chickens (Reese et al., 2013).

Antidiabetic peptides

Type 2 diabetes is a metabolic disorder characterized by a high concentration of glucose in the blood, derived from insulin resistance and relative insulin deficiency. To counteract this condition, intestinal L-cells secret glucagon-like peptide 1 (GLP-1), an incretin hormone, to stimulate insulin secretion from the pancreas in a glucose-dependent manner. In this sense, the oral delivery of GLP-1, through engineered probiotics, represents a promising strategy for the control of glucose concentration in a diabetic condition (Lin, Krogh-Andersen, Pelletier, Marcotte, Östenson, & Hammarström, 2016).

Thus, the engineered potential probiotic *L. lactis* FI5876 was designed to produce and deliver GLP-1, showing an improvement in glucose tolerance in mice administrated with a high-fat diet (Arora et al., 2016). Similarly, engineered *Lb. gasseri* ATCC 33323, capable to secrete GLP-1, showed a reduction of blood glucose levels (up to 33%) when was orally administrated to diabetic rats (Duan, Liu, & March, 2015).

In related work, oral administration of penetratin-fused GLP-1, produced by engineered *B. longum* HB15, increased the GLP-1 level in the colon, while the designed probiotic *Lb. paracasei* ATCC 27,092 secreting angiotensin (1–7) (Ang-(1–7)), increased the circulating levels of Ang-(1–7) (a vasodilator, angiogenic and anti-inflammatory peptide), and reduced the diabetic harmful effects on retina and kidney, as result of an increase in insulin production level after oral consumption (Wei et al., 2015; Carter et al., 2020; Verma et al., 2020).

Lb. paracasei BL23 was also engineered to produce GLP-1 by anchoring pentameric GLP-1 analogs to the bacterial surface. In a monomeric form, the bacterial GLP-1 improved glycemic control in diabetic rats; however, its efficacy as an insulinotropic alternative is still limited (Lin et al., 2016). In addition to the production of GLP-1, the secretion of proinsulin antigen along with immunomodulatory cytokine IL-10 has also been induced in the engineered *L. lactis* MG1363, which allowed reversal of established autoimmune diabetes in non-obese diabetic mice (Takiishi et al., 2012). In humans, this method could be effective for the treatment of type 1 diabetes.

Antihypertensive peptides

Hypertension (high blood pressure) is defined as a systolic blood pressure value of $\geq$ 140 mmHg and a diastolic pressure of $\geq$ 90 mmHg (140/90) in young persons, while for elderly persons $\geq$ 60 years, blood pressure values of above 160/90 mmHg may require treatment (Anker et al., 2021). Hypertension may be associated with pregnancy or diseases such as obesity, hypercholesterolemia, inflammation, and sleep apnea. The renin-angiotensin system is the most important metabolic pathway to control hypertension. Specifically, angiotensin-converting enzyme (ACE) is involved in the increase of blood pressure, as it converts angiotensin I into angiotensin II, a potent vasoconstrictor, and hydrolysates vasodilator peptides such as bradykinin and kallidin (Beltrán-Barrientos, Hernández-Mendoza, Torres-Llanez, González-Córdova, & Vallejo-Cordoba, 2016; Daliri, Lee & Oh, 2017).

Lifestyle modifications and pharmacological treatment have been recommended to prevent or reduce hypertension; however, the use of probiotics and their metabolites have been reported as promising alternatives to this objective. The antihypertensive effect of probiotics has been attributed to bioactive peptides with ACE-inhibitory activity. Antihypertensive peptides are difficult to purify, and their consistent production by probiotics is not guaranteed. Therefore, novel studies have focused on the use of engineered probiotics to express ACE-inhibitory peptides (Beltrán-Barrientos et al., 2021; Hag & Poondla, 2021). Yang et al. (2015), engineered probiotic *Lb. plantarum* NC8 with pSIP409 plasmid-bearing ACE-inhibitory peptides YFP and TFP, originally obtained from yellowish sole (*Limanda aspera*). The authors reported that rats fed with the engineered probiotic had significantly reduced systolic blood pressure for at least 10 days in comparison to the control groups, fed with conventional *Lb. plantarum* NC8 and phosphate buffer solution.

In another study, engineered *Lb. helveticus* LBK16H was designed to produce antihypertensive tripeptides, Ile-Pro-Pro and Val-Pro-Pro, from casein hydrolysis in fermented dairy products (Raveschot et al., 2018). Similarly, Losurdo, Quintieri, Caputo, Gallerani, Mayo, and De Leo (2013), engineered *Bifidobacterium pseudocatenulatum* M115, through the insertion of encoding synthetic genes, for the production of the hypertensive ACE-inhibitors peptides (FAQTQSLVPFPGPI, NIPPLTQTPV, and DKIHPF) and their bioactive precursors (FAQTQSLVYPFPG-PIPNSLP, NSLPQ-NIPPLTQTPVVVPPF, and EDELQDKIHPFAQTQS) respectively, from bovine β-casein hydrolysis.

Safety aspects and regulatory controls

Considering that engineered probiotics are not naturally occurring bacteria, the employment of biocontainment systems (either active or passive) should be included to prevent and or control the spread of these microorganisms into the environment (Plavec & Berlec, 2019; Hag & Poondla, 2021). According to the National Institutes of Health Benchmarks, the escape rate must be fewer than 1 in 10^8 recombinant or synthetic DNA molecules via either survival of the organisms or transmission to another organism (National Institutes of Health (NIH), 2016). Active biocontainment systems are designed to inhibit the engineered probiotic through the activation of killing gen or suppression of essential gen based on the strict stimulation by an environmental element. In contrast, the passive systems are based on the complementation of an auxotrophy or gene defect triggered by the supplementation of another gen or essential metabolite abnormal for the environment. The limitation of passive systems is that they are usually bacteriostatic rather than bactericidal (Plavec & Berlec, 2020).

According to current legislation, engineered probiotics are classified as genetically modified organisms; therefore, they are subject to strict regulations once they are destined for human consumption (Markakiou et al., 2020). The safety of engineered probiotics administration is related to the intended application, and some factors must be considered, including dosage duration of consumption, and consumer vulnerability. Indeed, clinical studies using engineered probiotics must be conducted and the deliberated release of the microorganism into an *in vivo* model must be addressed, ensuring adequate guidelines for its environmental biocontainment and subsequent eradication from the host (Kota, Ambati, Yalakurthi, Srirama, & Prakash, 2018; Hag & Poondla, 2021).

Engineered probiotics might act as reservoirs of antibiotic resistance

genes (Borrero et al., 2015). If this resistance gene transfer is vertical, it would not represent a safety problem itself. However, external factors can induce the horizontal transfer of resistance genes to another neighboring pathogenic bacterium, either pathogenic, probiotic, or commensal, by transduction (e.g., bacteriophages) or by the transformation between microorganisms (e.g., release DNA taken by another microorganism) (Álvarez-Cisneros & Ponce-Alquicira, 2018).

Thus, stability of genetic information is a prerequisite to use new engineered probiotics both in food products, as an ingredient or raw material, and in therapeutic approaches as cell factories of bioactive compounds (Plavec & Berlec, 2019, 2020). These regulatory features contribute to the correct design, production, and use of engineered probiotics and their bioactive metabolites, in concordance to the current international normativity and limitations according to their efficacy, risks, and mechanisms of action (Plavec & Berlec, 2019).

Future therapeutic uses of engineered probiotics

Scientists are interested in the use of engineered probiotics to improve and expand their therapeutic potential. In this sense, synthetic biology techniques have been used for the expression of specific genes for the subsequent synthesis of bioactive peptides with potential food and therapeutic functions (Plavec & Berlec, 2019). Synthetic biology techniques such as genetic engineering of microbes by cloning and overexpressing (using promoters, enhancers, and terminators), are considered important tools for researching and programming cellular behavior of microbes, including the characterization of relationships between microbes, host, and diseases (Yadav & Shukla, 2019). Therefore, probiotics can be designed as therapeutic agents for targeted drug delivery, as well as to restore homeostasis within a disturbed microbial community in the gut (Bober et al., 2018; Scheller, Strittmatter, Fuchs, Bojar, & Fussenegger, 2018).

One of the promising progress in microbial engineering is the transformation of bacteria species into genomic tape recorders or biosensors, capable to store information of physiological events or to respond to specific stimuli from the intestinal tract, such as inflammation, metabolic imbalance, or pathogen detection (Rottinghaus, Amrofell, & Moon, 2020). These approaches could increase the knowledge about the relationships between ecological changes inside GIT microbiota and epithelial barrier, and their connection to human health. As an example, Huang et al. (2016), engineered the cells of *E. coli* strains MC4100, TOP10, and MG1655 to sense high concentrations of *N*-acyl homoserine lactone (NHL), related to high cell density. In this study, engineered *E. coli* strains exhibited antibiotic resistance, allowing them to survive the antibiotic activity, while other microorganisms were inhibited. Antibiotic activity decreased cells density, in turn, NHL levels decreased sufficiently to signal to the engineered bacteria that the microbial effect has ended and then decreases their antibiotic resistance without transferring it to other microorganisms.

Although this effect cannot be applied *in vivo* systems, due to the antibiotic-based killing mechanism, another biosensor model based on quorum sensing sensibilization in an *in vivo* system was reported by Chowdhury et al. (2019). In this model, the authors demonstrated a localized release of immunotherapeutics and an abscopal effect of an engineered non-pathogenic *E. coli* using a mouse tumor model. These results contribute to the wide range of future applications in which microbial engineering can be applied with respect to the genetic modification of non-pathogenic bacteria for therapeutic purposes.

It has been reported that bioactive peptides from engineered probiotics may not exert the same beneficial effect on humans around the world (Daliri et al., 2018). For instance, Fekete, Givens, and Lovegrove, (2015), observed that antihypertensive lactotripeptides were effective in reducing blood pressure in Japanese but not in European patients. According to these findings, several authors recommend the development of *in vivo* and large-scale clinical trials to investigate the potency, efficacy, and safety properties of bioactive peptides, as well as their effects on higher population groups with different eating habits or clinical disorders, in order to expand the knowledge related to stability and functionality of bioactive peptides subjected to dynamic and real environments.

Conclusions

The use of synthetic biology tools offers a wide research niche in relation to the use of conventional probiotics for the obtention of new engineered microorganisms with increasingly specific positive effects, including high survival rates under harsh environmental conditions, improved metabolic systems, and potential use as therapeutic biological systems. The present review highlights the potential of synthetic biology tools for the development of new probiotics strains with improved benefits, including antimicrobial, antiviral, antidiabetic, and antihypertensive activities, based on the induced generation of predicted bioactive peptides. Based on the current information, beneficial effects are mostly evaluated by using *in vitro* and animal assays, which generates a lack of knowledge about the efficacy of these bioactive peptides in humans. In this sense, clinical trials are also needed to expand knowledge of the therapeutic capacity of bioactive compounds in populations from different geographical areas and with diverse lifestyles. On the other hand, the biosecurity of engineered probiotics and their bioactive compounds seems to be the main limitation for using human trials.

Although engineered probiotics may display certain benefits over conventional probiotics, it is necessary a better understanding of mechanisms underlying the health benefits. In addition, the interactions of engineered microorganisms with host cells and possible immune responses needed to be better described. Due to engineered probiotics being considered genetically modified organisms, the international safety recommendations and limitations for their use must be considered. Therefore, future research should be focused on the generation of new knowledge that contributes to the employment of engineered probiotics and potentially probiotics LAB, as cell factories to induce the selective generation of bioactive peptides with therapeutic and clinical functions with characterized safety parameters and improved acceptance among consumers.

CRediT authorship contribution statement

Haydee Eliza Romero-Luna: Investigation, Visualization, Writing – original draft. **Adrián Hernández-Mendoza:** Conceptualization, Writing – review & editing. **Aaron Fernando González-Córdova:** Writing – review & editing. **Audry Peredo-Lovillo:** Conceptualization, Writing – original draft, Writing – review & editing, Supervision.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

Aggarwal, N., Breedon, A. M. E., Davis, C. M., Hwang, I. Y., & Chang, M. W. (2020). Engineering probiotics for the therapeutic applications: Recent examples and translational outlook. *Current Opinion in Biotechnology, 65*, 171–179. https://doi.org/10.1016/j.copbio.2020.02.016

Ali, A., Kamal, M., Rahman, H., Siddiqui, N., Haque, A., Saha, K. K., & Rahman, A. (2021). Functional dairy products as a source of bioactive peptides and probiotics: Current trends and future prospects. *Journal of Food Science and Technology, 1–17*. https://doi.org/10.1007/s13197-021-05091-8

Álvarez-Cisneros, Y. M., & Ponce-Alquicira, E. (2018). Antibiotic resistance in lactic acid bacteria. In Y. Kumar (Ed.), *Antimicrobial Resistance-A Global Threat* (pp. 53–73). London, UK: Intech Open. https://doi.org/10.5771/intechopen.80624.

Anker, D., Santos-Eggimann, B., Zwahlen, M., Santschi, V., Rodondi, N., Wolfson, C., & Chiolero, A. (2021). Blood pressure control and complex health conditions in older adults: Impact of recent hypertension management guidelines. *Journal of Human Hypertension, 35*, 280–291. https://doi.org/10.1038/s41371-020-0334-4

Arora, T., Wegmann, U., Bobhate, A., Lee, Y. S., Greiner, T. U., Drucker, D. J., … Back, F. (2016). Microbially produced glucagon-like peptide1 improves glucose tolerance in mice. *Molecular Metabolism, 5*, 725–730. https://doi.org/10.1016/j.molmet.2016.06.006

T. Ayichew A. Belete T. Alebachew H. Tsehaye H. Bernahu A. Minwuyelet Bacterial probiotics their importances and limitatios: A review Journal of Nutrition and Health Sciences 4 2017 Article 202.

Beltrán-Barrientos, L. M., Garcia, H. S., Hernández-Mendoza, A., González-Córdova, A. F., & Vallejo-Cordoba, B. (2021). *Invited review:* Effect of antihypertensive fermented milks on gut microbiota. *Journal of Dairy Science, 104*, 3779–3788. https://doi.org/10.3168/jds.2020.19466

Beltrán-Barrientos, L. M., Hernández-Mendoza, A., Torres-Llanez, M. J., González-Córdova, A. F., & Vallejo-Cordoba, B. (2016). *Invited review*: Fermented milk as antihypertensive functional food. *Journal of Dairy Science, 99*, 4099–4110. https://doi.org/10.3168/jds.2015-10054

Bober, J. R., Beisel, C. L., & Nair, N. U. (2018). Synthetic biology approaches to engineer probiotics and members of the human microbiota for biomedical applications. *Annual Review of Biomedical Engineering, 20*, 277–300. https://doi.org/10.1146/annurev-bioeng-062117-121019

Borrero, J., Chen, Y., Dunny, G. M., & Kaznessis, Y. N. (2015). Modified lactic acid bacteria detect and inhibit multiresistant enterococci. *ACS Synthetic Biology, 4*, 299–306. https://doi.org/10.1021/sb500090b

Bradley, R. W., Buck, M., & Wang, B. (2016). Tools and principles for microbial gene circuit engineering. *Journal of Molecular Biology, 428*, 862–888. https://doi.org/10.1016/j.jmb.2015.10.004

Braff, D., Shis, D., & Collins, J. J. (2016). Synthetic biology platform technologies for antimicrobial applications. *Advanced Drug Delivery Reviews, 105*, 35–43. https://doi.org/10.1016/j.addr.2016.04.006

Bron, P. A., Marcelli, B., Mulder, J., van de Erls, S., Morawska, L. P., Kuipers, O. P., … Kleerebezem, M. (2019). Renaissance of traditional DNA transfer strategies for improvement of industrial lactic acid bacteria. *Current Opinion in Biotechnology, 56*, 61–68. https://doi.org/10.1016/j.copbio.2018.09.004

Carter, C. S., Morgan, D., Verma, A., Lobaton, G., Aquino, V., Sumners, E., … Buford, T. W. (2020). Therapeutic delivery of Ang(1–7) via genetically modified probiotic: A dosing study. *Journals of Gerontology: Biological Sciences, 75*, 1299–1303. https://doi.org/10.1093/gerona/glz222

Chai, K. F., Voo, A. Y. H., & Chen, W. N. (2020). Bioactive peptides from food fermentation: A comprehensive review of their sources, bioactivities, applications, and future development. *Comprehensive Reviews in Food Science and Technology, 19*, 3825–3885. https://doi.org/10.1111/1541-4337.12651

Chakrabarti, S., Guha, S., & Majumder, K. (2018). Food-derived bioactive peptides in human health: challenges and opportunities. *Nutrients, 10*, Article 1738. https://doi.org/10.3390/nu10111738.

Chen, S., Huang, G., Liao, W., Gong, S., Xiao, J., Bai, J., … Wu, J.-L. (2021). Discover of the bioactive peptides secreted by *Bifidobacterium* using integrated MCX coupled with LC-MS and featured-based molecular networking. *Food Chemistry, 347*, Article 129008. https://doi.org/10.1016/j.foodchem.2021.129008

Choudhury, A., Ortiz, P., & Kearney, C.M. (2021). *In vitro* of *H. pylori* in a preferential manner using bioengineered *L. lactis* releasing guided antimicrobial peptides. *bioRxiv*. https://doi.org/10.1101/2021.06.11.448139.

Chowdhury, S., Castro, S., Coker, C., Hinchliffe, T. E., Arpaia, N., & Danino, T. (2019). Programable bacteria induce durable tumor regression and systemic antitumor immunity. *Nature Medicine, 25*, 1057–1063. https://doi.org/10.1038/s41501-019-0498-z

Chua, K. J., Kwok, W. C., Aggarwal, N., Sun, T., & Chang, M. W. (2017). Designer probiotics for the prevention and treatment of human diseases. *Current Opinion in Chemical Biology, 40*, 8–16. https://doi.org/10.1016/j.cbpa.2017.04.011

Chugh, B., & Kamal-Eldin, A. (2020). Bioactive compounds produced by probiotics in food products. *Current Opinion in Food Science, 32*, 76–82. https://doi.org/10.1016/j.cofs.2020.02.003

Daliri, E.-B.-M., Lee, B. H., & Oh, D. H. (2017). Current perspective on antihypertensive probiotics. *Proteins & Antimicrobial Peptides, 9*, 91–101. https://doi.org/10.1007/s12602-016-9241-y

Daliri, E.-B.-M., Lee, B. H., & Oh, D. H. (2018). Current trends and perspectives of bioactive peptides. *Critical Reviews in Food Science and Nutrition, 58*, 2273–2284. https://doi.org/10.1080/10408398.2017.1319795

d'Ettorre, G., Ceccarelli, G., Marazzato, M., Campagna, G., Pinacchio, C., Alessandri, F., … Mastroianni, C. M. (2020). Challenges in the management of SARS-CoV2 infection: The role of oral bacteriotherapy as complementary therapeutic strategy to avoid the progression of COVID-19. *Frontiers in Medicine, 7*, 389. https://doi.org/10.3389/fmed.2020.00389

Delgado-Venegas, C. S., Martínez-Hernández, S. L., Cervantes-García, D., Montes de Oca-Luna, R., Loera-Arias, M. J., Mata-Martínez, M. G., … Muñoz-Ortega, M. H. (2021). Modulating effects of the probiotic *Lactococcus lactis* on the hepatic fibrotic induced by CCl_4 in Wistar rats. *Experimental and Therapeutical Medicine, 21*, 339. https://doi.org/10.3892/etm.2021.9770

Duan, F. F., Liu, J. H., & March, J. C. (2015). Engineered commensal bacteria reprogram intestinal cells into glucose-responsive insulin-secreting cells for the treatment of diabetes. *Diabetes, 64*, 1794–1803. https://doi.org/10.2337/db14-0635

Evivie, S. E., Huo, G.-C., Igene, J. O., & Bian, X. (2017). Some current applications, limitations and future perspectives of lactic acid bacteria as probiotics. *Food & Nutrition Research, 61*, 318034. https://doi.org/10.1080/16546628.2017.1318034

Fekete, A. A., Givens, D. I., & Lovegrove, J. A. (2015). Casein-derived lactotripeptides reduce systolic and diastolic blood pressure in a metanalysis of randomised clinical trials. *Nutrients, 7*, 659–681. https://doi.org/10.3390/nu7010659

Geldart, K., Borrero, J., & Kaznessis, Y. N. (2015). Chloride-inducible expression vector for delivery of antimicrobial peptides targeting antibiotic-resistant *Enterococcus faecium*. *Applied and Environmental Microbiology, 81*, 3889–3897. https://doi.org/10.1128/AEM.00227-15

Geldart, K. G., Komminer, S., Forbes, M., Hayward, M., Dunny, G. M., Salzman, N. H., & Kaznessis, Y. N. (2018). Engineered *E. coli* Nissle 1917 for the reduction of vancomycin-resistant *Enterococccus* in the intestinal tract. *Bioengineering & Tranlational Medicine, 3*, 197–208. https://doi.org/10.1002/btm2.10107

Hafeez, Z., Cakir-Kiefer, C., Roux, E., Perrin, C., Miclo, L., & Dary-Mourot, A. (2014). Strategies of producing bioactive peptides from milk proteins to functionalize fermented milk products. *Food Research International, 63*, 71–80. https://doi.org/10.1016/j.foodres.2014.06.002

Hag, S., & Poondla, N. (2021). Genetically engineered probiotics. In I. P. Kaur, & P. K. Deol (Eds.), *Probiotic Research in Therapeutics* (pp. 295–328). Singapore: Springer. https://doi.org/10.1007/978-981-15-8214-1_14.

Hidalgo-Cantabrana, C., O'Flaherty, S., & Barragou, R. (2017). CRISPR-based engineering of next-generation lactic acid bacteria. *Current Opinion in Microbiology, 37*, 79–87. https://doi.org/10.1016/j.mib.2017.05.015

Huang, S., Lee, A. J., Tsoi, R., Wu, F., Zhang, Y., Leong, K. W., & You, L. (2016). Coupling spatial segregation with synthetic circuits to control bacterial survival. *Molecular Systems Biology, 12*, Article 859. https://doi.org/10.15252/msb.20156567.

Huibregtse, I. L., Marietta, E. V., Rashtak, S., Koning, F., Rottiers, P., David, C. S., … Murray, J. A. (2009). Induction of antigen-specific tolerance by oral administration of *Lactococcus lactis* delivered immunodominant DQ8-restricted gliadin peptide in sensitized nonobese diabetic Ab° Dq8 transgenic mice. *Journal of Immunology, 183*, 2390–2396. https://doi.org/10.4049/jimmonol.0802891

Huynh, E., & Li, J. (2015). Generation of *Lactococcus lactis* capable of coexpressing epidermal growth factor and trefoil factor to enhance *in vivo* wound healing. *Applied Microbiology and Biotechnology, 99*, 4667–4677. https://doi.org/10.1007/s00253-015-6542-0

Hwang, I. Y., Koh, E., Wong, A., March, J. C., Bentley, W. E., Lee, Y. S., & Chang, M. W. (2017). Engineered probiotic *Escherichia coli* can eliminate and prevent *Pseudomonas aeruginosa* gun infection in animal models. *Nature Communications, 8*, 15208. https://doi.org/10.1038/ncomms15028

Jawan, R., Abbasiliasi, S., Mustafa, S., Kapri, M. R., Halim, M., & Ariff, A. B. (2020). In vitro evaluation of potential probiotic strain *Lactococcus lactis* Gh1 and its bacteriocin-like inhibitor substances for potential use in the food industry. *Probiotics and Antimicrobial Proteins, 13*, 422–440. https://doi.org/10.1007/s12602-09690-3

Jiménez, J. J., Diep, D. B., Borrero, J., Gútiez, L., Arbulu, S., Nes, I. F., … Hernández, P. E. (2015). Cloning strategies for heterologous expresion of the bacteriocin enterocin A by *Lactobacillus sakei* Lb790, *Lb. plantarum* NC8 and *Lb. casei* CECT475. *Microbial Cell Factories, 14*, 166. https://doi.org/10.1186/s12934-015-0346-x

Kohl, H., Castillo, A. R., & Ochoa-Repáraz, J. (2020). The microbiome as a therapeutic target for multiple sclerosis: Can genetically engineering probiotics treat the disease? *Diseases*, 8. *Article, 33*. https://doi.org/10.3390/diseases8030033

Kothari, D., Patel, S., & Kim, S. K. (2019). Probiotic supplements might not be universally-effective and safe: A review. *Biomedicine & Pharmacotherapy, 111*, 537–547. https://doi.org/10.1016/j.biopha.2018.12.104

Lagenaur, L. A., Sanders-Beer, B. E., Brichacek, B., Pal, R., Liu, X., Yu, R., … Hamer, D. H. (2011). Prevention of vaginal SHIV transmission in macaques by alive recombinant *Lactobacillus*. *Mucosal Immunology, 4*, 648–657. https://doi.org/10.1038/mi.2011.30

Li, R., Wan, X., Takala, T. M., & Saris, P. E. J. (2021). Heterologous expression of the *Leuconostoc* bacteriocin Leucocin C in probiotic yeast *Saccharomyces boulardii*. *Probiotics and Antimicrobial Proteins, 13*, 229–237. https://doi.org/10.1007/s12602-020-09676-1

Liang, W., Feng, Z., Rao, S., Xiao, C., Xue, X., Lin, Z., … Qi, W. (2020). Diarrhoea may be underestimated: A missing link in 2019 novel coronavirus. *Gut, 69*, 1141–1143. https://doi.org/10.1136/gutjnl-2020-320832

Limaye, S. A., Haddad, R. I., Cilli, F., Sonis, S. T., Colevas, D., Brennan, M. T., … Murphy, B. A. (2013). Phase 1b, multicenter, single blinded, placebo-controlled, sequential dose escalation study to assess the safety and tolerability applied AGO13 in subjects with locally advanced head and neck cancer receiving induction chemotherapy. *Cancer, 119*, 4268–4276. https://doi.org/10.1002/cncr.28365

Lin, Y., Krogh-Andersen, K., Pelletier, J., Marcotte, H., Östenson, C.-G., & Hammarström, L. (2016). Oral delivery of pentameric glucagon-like peptide-1 by recombinant *Lactobacillus* in diabetic rats. *PLoS ONE, 11*, e0162733. https://doi.org/10.1371/journal.pone.0162733

Liu, M., Zhang, X., Hao, Y., Ding, J., Shen, J., Xue, Z., … Wang, N. (2019). Protective effects of a novel probiotic strain, *Lactococcus lactis* ML2018, in colitis: *In vivo* and *in vitro* evidence. *Food & Function, 10*, 1132–1145. https://doi.org/10.1039/C8FO02301H

Losurdo, L., Quintieri, L., Caputo, L., Gallerani, R., Mayo, B., & De Leo, F. (2013). Cloning and expression of synthetic genes encoding angiotensin-I converting enzyme (ACE)-inhibitory bioactive peptides in *Bifidobacterium pseudocatenulatum*. *FEMS Microbiology Letters, 340*, 24–32. https://doi.org/10.1111/1574-6968.12068

Kasarello, K., Szczepankowska, A., Kwiatkowska-Patzer, B., Lipkowski, A. W., Gadamski, R., Sulejczak, D., … Bardowski, J. (2016). Effect of recombinant *Lactococcus lactis* producing myelin peptides on neuroimmunological changes in rats with experimental allergic encephalomyelitis. *Folia Neuropathologica, 54*, 249–258.

Kota, R. K., Ambati, R. R., Yalakurthi, A. K., Srirama, K., & Prakash, N. R. (2018). Recent advances in probiotics as live biotherapeutics against gastrointestinal diseases. *Current Pharmaceutical Desing, 24*, 1–9. https://doi.org/10.2174/1381612824666180717105128

Kumar, M., Yadav, A. K., Verma, V., Singh, B., Mal, G., Nagpal, R., & Hemalatha, R. (2016). Bioengineered probiotics as a new hope for health and diseases: An overview

of potential and prospects. *Future Microbiology, 11*, 585–600. https://doi.org/10.2217/fmb.16.4
Mandal, S. M., Pati, B. R., Chakraborty, R., & Franco, O. L. (2016). New insights into the bioactivity of peptides from probiotics. *Frontiers in Bioscience, 8*, 450–459. https://doi.org/10.2741/E779
Mandal, S. M., Silva, O. N., & Franco, O. L. (2014). Recombinant probiotics with antimicrobial peptides: A dual strategy to improve immune response in immunocompromised patients. *Drug Discovery Today, 19*, 1045–1050. https://doi.org/10.1016/j.drudis.2014.05.019
Mansour, N. M., & Abdelaziz, S. A. (2016). Oral immunization of mice with engineered *Lactobacillus gasseri* NM713 strain expressing *Streptococcus pyogenes* M6 antigen. *Microbiology and Immunology, 60*, 527–532. https://doi.org/10.1111/1348-0421.12397
Markakiou, S., Gaspar, P., Johansen, E., Zeidan, A. A., & Neves, A. R. (2020). Harnessing the metabolic potential of *Streptococcus thermophilus* for new biotechnological applications. *Current Opinion in Bacteriology, 61*, 142–152. https://doi.org/10.1016/j.copbio.2019.12.019
Martínez-Fernández, J. A., Bravo, D., Peirotén, Á., Arqués, J. L., & Landete, J. M. (2019). Bile-induced promoters for gene expression in *Lactobacillus* strains. *Applied Microbiology and Biotechnology, 103*, 3819–3827. https://doi.org/10.1007/s00253-019-09743-w
Mathipa, M. G., & Thantsha, M. S. (2017). Probiotic engineering: Towards development of robust probiotic strains with enhanced functional properties and for targeted control of enteric pathogens. *Gut Pathogens, 9*, 1–17. https://doi.org/10.1186/s13099-017-0178-9
Mays, Z. J. S., & Nair, N. U. (2018). Synthetic biology in probiotic lactic acid bacteria: At the frontier of living therapeutics. *Current Opinion in Biotechnology, 53*, 224–231. https://doi.org/10.1016/j.copbio.2018.01.028
Mazhar, S. F., Afzal, M., Almatroudi, A., Munir, S., Ashfaq U. A., Rasool, M., Raza, H., Munir, H. M. W., Rajoka, M. S. R., & Khurshid, M. (2020). The prospects for the therapeutic implications of genetically engineered probiotics. *Journal of Food Quality*, *2020*, Article 9676452. https://doi.org/10.1155/2020/9676452.
Mejía-Pitta, A., Broset, E., & de la Fuente-Nunez, C. (2021). Probiotic engineering strategies for the heterologous production of antimicrobial peptides. *Advanced Drug Delivery Reviews, 176*, Article 113863. https://doi.org/10.1016/j.addr.2021.113863
Moradi-kalbolandi, S., Majidzadeh-A, K., Abdolvahab, M. H., Jalili, N., & Farahmand, L. (2021). The role of mucosal immunity and recombinant probiotics is SARS-CoV-2 vaccine development. *Probiotics and Antimicrobial Proteins, 13*, 1239–1253. https://doi.org/10.1007/s12602-021-09773-9
Nguyen, H.-T., Truong, D.-H., Kouhoundé, S., Ly, S., Razafindralambo, H., & Delvigne, F. (2016). Biochemical engineering approaches for increasing viability and functionality of probiotic bacteria. *International Journal of Molecular Sciences, 17*, 867. https://doi.org/10.3390/ijms17060867
National Institutes of Health (NIH) (2019). NIH guidelines for research involving recombinant or synthetic nucleic acids molecules. Bethesda, MD, USA. https://https://osp.od.nih.gov/wp-content/uploads/NIH_Guidelines.pdf. Accessed August 4, 2021.
Piñero-Lambea, C., Ruano-Gallego, D., & Fernández, L.Á. (2015). Engineered bacteria as therapeutic agents. *Current Opinion in Biotechnology, 35*, 94–102. https://doi.org/10.1016/j.copbio.2015.05.004
Plavec, T. V., & Berlec, A. (2019). Engineering of lactic acid bacteria for delivery of therapeutic proteins and peptides. *Applied Microbiology and Biotechnology, 103*, 2053–2066. https://doi.org/10.1007/s00253-019-09628-y
Plavec, T. V., & Berlec, A. (2020). Safety aspects of genetically modified lactic acid bacteria. *Microorganisms, 8*, 297. https://doi.org/10.3390/microorganisms8020297
Plaza-Diaz, J., Ruiz-Ojeda, F. J., Gil-Campos, M., & Gil, A. (2019). Mechanisms of Action of Probiotics. *Advances Nutrition, 10*(suppl_1), S49–S66. https://doi.org/10.1093/advances/nmy063
Rao, S., Hu, S., McHugh, L., Lueders, K., Henry, K., Zhao, Q., … Hamer, D. H. (2005). Toward a live microbial microbicide for HIV: Commensal bacteria secreting an HIV fusion inhibitor peptide. *Proceedings of the National Academy of Sciences, 105*, 11993–11998. https://doi.org/10.1073/pnas0504881102
Raveschot, C., Cudennec, B., Coutte, F., Flahaut, C., Fremont, M., Drider, D., & Dhulster, P. (2018). Production of bioactive peptides by *Lactobacillus* species: From gene to application. *Frontiers in Microbiology, 9*, 2354. https://doi.org/10.3389/fmicb.2018.02354
Reese, K. A., Lupfer, C., Johnson, R. C., Mitev, G. M., Mullen, V. M., Geller, B. L., & Pastey, M. (2013). A novel lactococcal vaccine expressing a peptide from the M2 antigen of H5N2 highly pathogenic avian influenza a virus prolongs survival of vaccinated chickens. *Veterinary Medicine International, 2013*, Article 316926. https://doi.org/10.1155/2013/316926
Renye, J. A., & Somkuti, G. A. (2008). Cloning of milk-derived bioactive peptides in *Streptococcus thermophilus*. *Biotechnology Letters, 30*, 723–730. https://doi.org/10.1007/s10529-007-9600-6
Riglar, D. T., & Silver, P. A. (2018). Engineering bacteria for diagnostic and therapeutic applications. *Nature Reviews Microbiology, 16*, 214–225. https://doi.org/10.1038/nrmicro.2017.172
Rottinghaus, A. G., Amrofell, M. B., & Moon, T. S. (2020). Biosensing in smart engineered probiotics. *Biotechnology Journal, 15*, 1900319. https://doi.org/10.1002/biot.2019003310
Sakaguchi, K., Funaoka, N., Tani, S., Kano, Y., & Suzuki, T. (2012). A targeted gene knockout method using a newly constructed temperature-sensitive plasmid mediated homologous recombination in *Bifidobacterium longum*. *Applied Microbiology and Biotechnology, 95*, 499–509. https://doi.org/10.1007/s00253-012-4090-4
Sánchez, A., & Vázquez, A. (2017). Bioactive peptides: A review. *Food Quality and Safety, 1*, 29–46. https://doi.org/10.1093/fqsafe/fyx006
Scheller, L., Strittmatter, T., Fuchs, D., Bojar, D., & Fussenegger, M. (2018). Generalized extracellular molecules sensor platform for programming cellular behavior. *Nature Chemical Biology, 14*, 723–729. https://doi.org/10.1038/s41589-018-0046-z
Senapati, S., Dash, J., Sethi, M., & Chakraborty, S. (2020). Bioengineering probiotics to control SARS-CoV-2 infection. *Research Ideas and Outcomes, 6*, e54802. https://doi.org/10.3897/rio.6.e54802
Shenderov, B., Sinitsa, A., Zakharchenko, M., & Lang, C. (2021). Drawbacks and Negative Consequences of Traditional Probiotics Based on Live Microorganisms. In: *METABIOTICS*. Springer, Cham. https://doi.org/10.1007/978-3-030-34167-1_9.
Škrlec, K., Ručman, R., Jarc, E., Sikirić, P., Švajger, U., Petan, T., … Berlec, A. (2018). Engineering recombinant *Lactococcus lactis* as a delivery vehicle for BPC-157 peptide with antioxidant activities. *Applied Microbiology and Biotechnology, 102*, 10103–10117. https://doi.org/10.1007/s00253-018-9333-6
Sola-Oladokun, B., Culligan, E. P., & Sleator, R. D. (2017). Engineered probiotics: Applications and biological containment. *Annual Review of. Science and Technology, 8*, 16.1-16.18. https://doi.org/10.1146/annurev-food-030216-030256
Son, J., & Jeong, K. J. (2020). Recent advances in synthetic biology for the engineering of lactic acid bacteria. *Biotechnology and Bioprocess Engineering, 25*, 962–973. https://doi.org/10.1007/s12257-020-0033-6
Sotoudegan, F., Daniali, M., Hassani, S., Nikfar, S., & Abdollahi, M. (2019). Reappraisal of probiotics' safety in human. *Food and Chemical Toxicology, 129*, 22–29. https://doi.org/10.1016/j.fct.2019.04.032
Taghinezhad-S, S., Mohseni, A. H., Bermúdez-Humarán, L. G., Casolaro, V., Cortes-Perez, N. G., Keyvani, H., & Simal-Gandara, J. (2021). Probiotic-based vaccines may provide effective protection against COVID-19 acute respiratory disease. *Vaccines, 9*, 499. https://doi.org/10.3390/vaccines9050466
Takiishi, T., Korf, H., Van Belle, T. M., Robert, S., Grieco, F. A., Caluwaerts, S., … Mathieu, C. (2012). Reversal of autoimmune diabetes by restoration of antigen-specific tolerance using genetically modified *Lactococcus lactis* in mice. *Journal of Clinical Investigation, 122*, 1717–1725. https://doi.org/10.1172/JCI60530
Taniguchi, M., Aida, R., Saito, K., Ochiai, A., Takesono, S., Saitoh, E., & Tanaka, T. (2018). Identification and characterization of multi-functional cationic peptides from traditional Japanese fermented soybean Natto extracts. *Journal of Bioscience and Bioengineering, 127*, 472–478. https://doi.org/10.1016/j.jbiosc.2018.09.016
van Pijkeren, J.-P., Neoh, K. M., Sirias, D., Findley, A. S., & Britton, R. A. (2012). Exploring optimization parameters to increase ssDNA recombineering in *Lactococcus lactis* and *Lactobacillus reuteri*. *Bioengineering, 3*, 209–217. https://doi.org/10.4161/bioe.21049
Veiga, P., Suez, J., Derrien, M., & Elinav, E. (2020). Moving from probiotics to precision probiotics. *Nature Microbiology, 5*, 878–880. https://doi.org/10.1038/s41564-020-0721-1
Verma, A., Zhu, P., Xu, K., Du, T., Liao, S., Liang, Z., … Li, Q. (2020). Angiotensin-(1–7) expressed from Lactobacillus bacteria protect diabetic retina mice. *Translational Vision. Science & Technology, 9*, 20. https://doi.org/10.1167/tvst.9.13.20
Walls, A., Park, Y., Tortorici, M. A., Wall, A., McGuire, A., & Veesler, D. (2020). Structure, function, and antigenicity of the SARS-CoV-2 spike glycoprotein. *Cell, 180*, 281–292. https://doi.org/10.1016/j.cell.2020.02.058
Wang, M., Fu, T., Hao, J., Li, L., Tian, M., Jin, N., … Li, C. (2020b). A recombinant *Lactobacillus plantarum* strain expressing the spike protein of SARS-CoV-2. *International Journal of Biological Molecules, 160*, 736–740. https://doi.org/10.1016/j.ijbiomac.2020.05.239
Wang, Q., Zhang, Y., Wu, L., Niu, S., Song, C., Zhang, Z., … Qi, J. (2020a). Structural and functional basis of SARS-CoV-2 entry by using 2 human ACE2. *Cell, 181*, 894–904. https://doi.org/10.1016/j.cell.2020.03.045
Wang, X., Wang, L., Huang, X., Ma, S., Yu, M., Shi, W., Qiao, X., Tang, L., Xu, Y., & Li, Y. (2017). Oral delivery of probiotics expressing dendritic cell-targeting peptide fused with porcine epidemic diarrhea virus COE antigen: Promising Vaccine Strategy Against PEDV. *Viruses*, *9*, Article e312. https://doi.org/doi.org/10.3390/v9110312.
Wei, P., Yang, Y., Ding, Q., Li, X., Sun, H., Liu, Z., … Gong, Y. (2016). Oral delivery of *Bifidobacterium longum* expressing α-melanocyte-stimulating hormone to combat ulcerative colitis. *Journal of Medical Microbiology, 65*, 160–168. https://doi.org/10.1099/jmm.0.000197
Wei, P., Yang, Y., Li, T., Ding, Q., & Sun, H. (2015). A engineered *Bifidobacterium longum* secreting a bioactive penetratin-Glucagon-like peptide 1 fusion protein enhances Glucagon-like peptide 1 absorption in the intestine. *Journal of Microbiology and Biotechnology, 2015*. https://doi.org/10.4014/jmb.1412.12030
Wong, C. C., Zhang, L., Wu, W. K., Shen, J., Chan, R. L., Lu, L., … Cho, C. H. (2017). Cathelicidin-encoding *Lactococcus lactis* promotes mucosal repair in murine experimental colitis. *Journal of Gastroenterology and Hepatology, 32*, 609–619. https://doi.org/10.1111/jgh.13499
Xia, P.-F., Ling, H., Foo, J. L., & Chang, M. W. (2019). Synthetic genetic circuits for programmable biological functionalities. *Biotechnology Advances, 37*, Article 107393. https://doi.org/10.1016/j.biotechadv.2019.04.015
Yadav, M., & Shukla, P. (2019). Efficient engineered probiotics using synthetic biology approaches: A review. *Biotechnology and Applied Biochemistry, 67*, 22–29. https://doi.org/10.1002/bab.1822
Yadav, R., Kumar, V., Baweja, M., & Shukla, P. (2018). Gene editing and genetic engineering for advanced probiotics: A review. *Critical Reviews in Food Science and Nutrition, 58*, 1735–1746. https://doi.org/10.1080/104083398.2016.1274877
Yang, G., Jiang, Y., Yang, W., Du, F., Yao, Y., Shi, C., & Wang, C. (2015). Effective treatment of hypertension by recombinant *Lactobacillus plantarum* expressing angiotensin converting enzyme inhibitor peptide. *Microbial Cell Factories, 14*, 202. https://doi.org/10.1186/s12934-015-0394-2
Zhang, B., Li, A., Zuo, F., Yu, R., Zeng, Z., Ma, H., & Chen, S. (2016a). Recombinant *Lactococcus lactis* NZ9000 secretes a bioactive kisspeptin that inhibits proliferation

and migration of human colon carcinoma HT-29 cells. *Human Cell Factories, 15*, 102. https://doi.org/10.1186/s12934-016-0506-7

Zhang, L., Will, W. K. K., Gallo, R. L., Fang, E. F., Hu, W., Ling, T. K. W., Shen, J., Chan, R. L. Y., Lu, L., Luo, X. M., Li, M., Chan, K. M., Yu, J., Wong, V. W. S., Ng, S. C., Wong, S. H., Chan, F. K. L., Sung, J. J. Y., Chan, M. T. V., & Cho, C. H. (2016b). Critical role of antimicrobial peptide cathelicidin for controlling *Helicobacter pylori* survival and infection. *Journal of Immunology, 196*, 1799–1809. https://doi.org/10.4049/jimmunol.1500021

Zhou, Z., Chen, X., Sheng, H., Shen, X., Sun, X., Yan, Y., … Yuan, Q. (2020). Engineering probiotics as living diagnostics and therapeutics for improving human health. *Microbial Cell Factories, 19*, 1–12. https://doi.org/10.1186/s12934-020-01318-z

Zmora, N., Zilberman-Schapira, G., Suez, J., Mor, U., Dori-Bachash, M., Bashiardes, S., … Elinav, E. (2018). Personalized gut mucosal colonization resistance to empiric probiotics is associated with unique host and microbiome features. *Cell, 174*(6), 1388–1405. https://doi.org/10.1016/j.cell.2018.08.041

Zuo, F., Chen, S., & Marcotte, H. (2020). Engineer probiotic bifidobacteria for food and biomedical applications-current status and future prospective. *Biotechnology Advances, 45*, Article 107654. https://doi.org/10.1016/j.biotechadv.2020.107654

Zuo, Y., & Chen, T. (2020). Engineered *Akkermansia muciniphila*: A promising agent against diseases (Review). *Experimental and Therapeutic Medicine, 20*, 285. https://doi.org/10.3892/etm.2020.9415