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Review Article

The Challenge of Applications of Probiotics in Gastrointestinal Diseases

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Gastrointestinal disease is characterized by gastrointestinal dysfunction with dysbiosis of the microbiome. Probiotics may act as biological agents in treating gastrointestinal diseases through modifying gut microbiota. However, several challenges, including safety, stress resistance, postcolonization quantification, and evaluation models, may hinder the application of probiotics in gastrointestinal diseases. This review introduces the emerging methods for delivering probiotics as well as available materials. Furthermore, we elucidated bacteriocins and their role in helping probiotics obtain a competitive advantage over other strains and challenges of large-scale application. Bacteriocins produced by probiotics also showed promising efficacy in gastrointestinal diseases including the capacity of immune stimulation, intestinal barrier protection, and cytotoxicity against intestinal tumorigenesis. For the quantification of probiotics in complex microbiomes and evaluation methods of probiotic encapsulated delivery systems, recent fluorescent labeling technology and various *in vitro* and *in vivo* models were also reviewed. Given the widespread use of probiotic agents in the microecological therapy of gastrointestinal diseases, further understanding of the multiple challenges of probiotic application and the updated methods to improve the colonization and evaluation system of probiotics is of great significance for probiotics as live biotherapeutics.

1. Introduction

Although probiotics exhibit high potential as therapeutic agents in treating gastrointestinal diseases, the applications of probiotics are still facing challenges [1]. Firstly, probiotics must be safe for human consumption, without any transferable anti-biotic-resistant genes [2]. Therefore, engineered probiotics are usually not allowed to be used for treatment of diseases. Secondly, the minimum viable counts ($\sim 10^6$ CFU/g) are required in order to be beneficial [3]. However, the most commonly used probiotics usually belong to *Lactobacillus* and *Bifidobacterium*, which are very susceptible to aerobic, and high-temperature environments, and the emerging next-generation probiotics demand more favorable conditions. Besides, probiotics must endure the stomach acid and bile during the gastrointestinal transition

[4]. Even though probiotics reach the colon alive, they may have to adhere to the mucus layer and colonize the colon in order to be effective. Therefore, design of probiotic formulations for targeted delivery to the intestines is quite challenging. Bacteriocins could act as a "colonizing peptide," "killing peptide," and "signal peptide" by promoting the colonization of the producing strain in the gut to gain a competitive advantage over other strains. Thirdly, in quantifying probiotics, many problems still need to be solved, such as on-site localization and dynamic monitoring. The existing feces examining methods cannot satisfy fast-developing gut microbiota studies [5]. Therefore, new tools are urgently needed, such as fluorescent labeling and imaging of gut microbes. Last but not least, establishment of more proper *in vitro* and *in vivo* models for evaluating the function of probiotics or probiotic encapsulated delivery systems is required. By solving these problems, probiotics may exhibit good prospects in the food and pharmaceutical fields in the future. Here, we discuss the potential therapeutic challenges of probiotic application and updated recent progress.

2. Targeted Delivery of Probiotics

With the ever-growing health needs of people, delivery of probiotics through dietary supplements, foods, and beverages has become increasingly popular. Such dietary supplements and nutritionally enhanced foods are appealing to different groups of people with high health care demand, such as women, infants, children, adolescents, the elderly, and those recovering from wounds or surgery [6]. The best-known functional foods with added probiotics include yogurt, cheese, ice cream, and other dairy products, while novel nondairy products emerged recently, and the market keeps expanding [7]. Moreover, novel snacks which have been incorporated with desired strain combinations include chocolate bars, cereal, juice, and chips [8].

Successful incorporation of probiotics into dietary supplements or foods requires careful experiments to find out optimum strain combination and formulation to deliver them. Challenges such as pH and water activity adjustment, temperature control, shelf-life evaluation, and sensory concerns need to be overcome before a perfect product can be achieved for consumers [9, 10]. Normally, low storage temperature and high-fat content are favorable conditions for the incorporation of probiotics, which make dairy products ideal candidates for probiotic-enhanced foods. Encapsulation and controlled release systems which are popular among pharmacists have been adopted to stabilize the probiotics in certain matrices of foods or beverages recently, resulting in more varieties of snacks containing healthy probiotics [11, 12].

Most recently, there has been an increased interest in targeted delivery of probiotics. Normally, such targeted delivery is aimed at getting the probiotics to the intestine so that better health benefits can be achieved, including enhancing gastrointestinal stability, reducing lactose intolerance, relieving diarrhea, boosting immunity, and lowering cholesterol [13–15].

The formulation of probiotics for targeted delivery to the intestines can be quite challenging in order to resist the strong acidic gastrointestinal environment. Exposure to gastric acid can be devastating to unprotected probiotics, and this is why coating layers and encapsulation techniques must be applied to protect the probiotics from such strong acidic environment until they are delivered to the targeted place [16]. The most commonly used encapsulation approaches are pH-sensitive and bacteria sensitive coating layers, which can release the coated probiotics in the intestine in response to specific pH conditions or certain bacteria colonies [17] (Figure 1). Other critical considerations include utilization of natural and economical coating materials; increasing the adhesion of the outer surface of the coating to epithelial cells of the intestines; enhancing the bioavailability, bile salt hydrolase activity, stability of the probiotics, antagonistic activity, and efficacy; and targeting capacity of the delivery and safety concerns related [10].

Various wall materials have been investigated to achieve the goal of targeted delivery of different probiotics, and the most widely used include dietary fibers, proteins, and natural polysaccharides [18]. Synthetic materials with good biocompatibility have been used as well. To achieve better efficacy, combinations of different wall materials have also been attempted, and emulsifiers are added as match makers. More importantly, the stability of the probiotics itself has to be taken into account, especially during the drying step of food processing [19]. Here, the drying techniques usually include freeze drying, spray drying, extrusion, refractance window drying, electrospraying, electrospinning, and emulsifying. The cost and the temperature of each drying process are important factors to choose a proper processing approach [6]. Additionally, advanced techniques such as microfluidics, genetic engineering, and 3D printing have also been employed to achieve enhanced encapsulation efficiency recently [20].

3. Bacteriocins: Advantages and Challenges

Bacteriocins are antimicrobial peptides synthesized in the ribosome [21], and many probiotic strains were found with a capacity of producing bacteriocins, like the strain *Lactococcus lactis* which can produce nisin [22, 23]. Bacteriocins are classified into four classes according to their molecular weight, chemical structure, thermal stability, and modification [24–26]. It is generally believed that bacteriocins kill the targeted cells by destroying the integrity of cell membranes [27], cracking bacterial DNA, interacting with intracellular enzymes, and interrupting bacterial protein synthesis [28, 29]. Class I bacteriocins are small posttranslationally modified ribosomal peptides, which is the class nisin belongs to [30] (Table 1). Class II bacteriocins are small thermostable peptides (5-10 kDa) and nonmodified proteins (such as pediocin PA-1 and plantaricin EF) [31, 32]. Class III bacteriocins are macromolecular heat-sensitive proteins (>30 kDa) (such as *Lactococcin* 972) [33]. Class IV bacteriocins consist of large complexes with carbohydrates or lipid moieties (such as *Lactocin* 27) [34]. In addition to the above

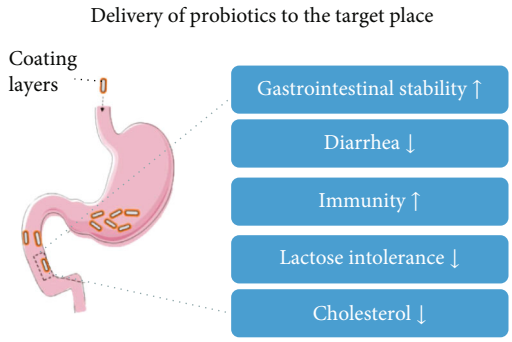

Figure 1: Delivery of probiotics to the target place.

Table 1: Bacteriocin types.

	Bacteriocin
Class I	Small posttranslationally modified ribosomal peptides
Class II	Small thermostable peptides, nonmodified proteins (5-10 kDa)
Class III	Macromolecular heat-sensitive proteins (>30 kDa)
Class IV	Large complexes with carbohydrate or lipid moieties
Class V	"Kemperman class V" bacteriocin

four class, Kemperman et al. proposed a separate class of cyclic bacteriocins—"Kemperman Class V" bacteriocin [35].

Bacteriocins and bacteriocin-producing probiotics are widespread in the gut. Drissi et al. reported that the *Firmicutes* (mainly *Streptococcus* and *Lactobacillus*), which dominate the human gut microbiota, can produce the largest number of bacteriocin (about 70.79%) [36]. Among them, the vast majority bacteria encoding bacteriocins belong to lactic acid bacteria (LAB), indicating that bacteriocins from LAB play a crucial role in maintaining the balance of gut microflora. Bacteriocins could act as a "colonizing peptide," "killing peptide," and "signal peptide" by promoting the colonization of the producing strain in the gut to gain a competitive advantage over other strains [23], targeting undesirable pathogens without negatively affecting beneficial flora [37], and participating in quorum sensing with other bacteria or to signaling cells in the host immune system, respectively. Pediocin PA-1, produced by *Pediococcus acidilactici* UL5, was reported to reduce the intestinal colonization of vancomycin-resistant enterococci (VRE) *in vivo* [38]. Garvicin ML, produced by *L. garvieae* DCC43, was able to improve host health by modifying intestinal microbiota, including potentially problematic bacteria inhibition, LAB proportion increase, and triglyceride level decrease [23].

Bacteriocins are involved in immune regulation and intestinal epithelial barrier protection, thus controlling diseases such as obesity (Table 2) [39, 40]. Nisin and CBP22 were found to protect the host from pathogens' infection by enhancing the intestinal immunity [41, 42]. Nisin could also affect neutrophils and induce the formation of neutrophil extracellular traps *in vitro* in a dose-dependent manner [41, 43]. Yin et al. reported that plantaricin EF can improve the symptoms of acute inflammatory bowel disease in mice by regulating the levels of TNF-α and IL-6 [44], and it also protects the epithelial barrier by increasing ZO-1 expression in the ileum in obese mice Heeney et al. [31].

Bacteriocins also appear to have selective cytotoxicity against colorectal cancer cells in comparison to normal cells [45], enabling inhibition of intestinal tumorigenesis. *In vitro* assays revealed that different concentration of nisin inhibited the proliferation of SW48, HT29, and Caco-2 cells to different degrees [46]. De Giani et al. reported that plantaricin P1053 can reduce the proliferation of intestinal cancer cells while enhancing the vitality of healthy cells [47]. In addition to its antitumor activity *in vitro*, bacteriocins also show good efficacy *in vivo* studies on tumor inhibition [48, 49]. Reutein is a class II bacteriocin isolated from *L. reuteri*. Bell et al. found that tumor volume of mice treated with reutein was significantly reduced when the nude mouse model was implanted by HCT116 and SW480 colon cancer xenograft [45]. Goyert et al. found that reuterin can inhibit the growth of colorectal cancer by altering the redox balance [50].

Bacteriocins have proved to be an important weapon in maintaining microecological balance and gut health [51]. However, there are certain challenges in bringing bacteriocins into large-scale application. Firstly, bacteriocins obtained by traditional separation methods such as genetic engineering to construct recombinant cells have low yields and take a long time, while chemical synthesis methods are expensive and not suitable for large-scale production [52]. Furthermore, another issue is the instability of bacteriocins under low/high pH and pepsin influenced conditions [51]. Meanwhile, the degree of expression of bacteriocins under harsh gastrointestinal conditions has not been elaborated, and further research is required to establish a method to improve the stability and efficacy of bacteriocin as biologics.

4. Quantification of Probiotics: Fluorescence Labeling

At present, the identification and quantification of most gastrointestinal microbiomes are highly dependent on the high-throughput DNA sequencing of fecal microflora [53]. The gut microbiota is composed in a certain proportion to form a stable ecosystem, where bacteria cooperate and restrain each other. It is controversial whether the fecal microbiome can represent the whole composition of the colonized bacteria in the gut [54, 55]. Simultaneously, many problems still need to be solved in quantifying probiotics, such as on-site localization and dynamic monitoring. Many foreign bacteria are excreted with the feces as they cannot colonize in the intestinal tract. The composition of bacteria in feces exceeds that of colonized gut microbiota. The existing feces examining methods cannot satisfy rapidly developing gut microbiota studies. Therefore, new tools are urgently needed to detect microbial communities and their presence in the digestive tract. Gut microbes' fluorescent labeling and imaging could be an appropriate method to resolve this problem.

Researchers have recently been devoted to developing convenient bacteria fluorescent imaging methodologies. Transferring fluorescent protein genes into bacteria genes

Table 2: Effect of bacteriocins on gastrointestinal health.

Bacteriocin	Class	Producing strain	Effect	References
Abp118	II	*L. salivarius* UCC118	Alleviate metabolic abnormalities associated with obesity	[39]
Pediocin PA-1	II	*P. acidilactici* PAC1.0	Reduce the intestinal colonization of VRE; inhibit the growth of DLD-1 and HT29	[38, 49, 96]
Garvicin ML	II	*L. garvieae* DCC43	Significantly increase the counts of total LAB and decrease the blood serum levels of triglycerides	[23]
Nisin	I	*L. lactis*	Regulate the intestinal immune; inhibit colorectal cancer in vitro	[43, 97, 98]
CBP22	I	*Clostridium butyricum* ZJU-F1	Prevention of LPS-induced gut barrier dysfunction by modulating the immune system	[42]
Plantaricin P1053	I	*L. plantarum* PBS067	Reduce proliferation of cancer-causing human intestinal cells	[47]
Reuterin	II	*L. reuteri*	Anticancer activity against HCT-116, SW480, RKO, and DLD-1 colorectal cancer cells	[45, 50]
Nisin A	I	*L. lactis*	Inhibit colorectal cancer cells LS180, SW48, HT29, and Caco-2	[99]
Pediocin K2a2-3	II	*P. acidilactici* K2a2-3	Inhibit the proliferation of HT29	[100, 101]
Bactofencin A	II	*L. salivarius* DPC6502	Alter the proportions of several important gut bacteria, such as *Fusobacterium*, *Bacteroides*, and *Bifidobacterium*	[102]
Plantaricin EF	II	*L. plantarum* 163	Ameliorate the effects of obesogenic diets; acute inflammatory bowel disease	[31, 44]
Gassericin A	V	*L. gasseri* LA39	Affect the differentiation and development of adipocytes	[103]

was initially attempted [56, 57]. However, most of the gut microbiota is hard to isolate and culture under artificial conditions, making the gene transfer challenging [58]. Thus, *in vitro* testing tools based on bacterial nucleic acid sequencing, such as fluorescence in situ hybridization (FISH), were adopted [59]. It is unfortunate that FISH can only label dead bacteria. Therefore, many attempts have been made to label bacteria *in vivo*.

Since some antibiotics can specifically bind to the bacterial outer membrane, antibiotics are conjugated with fluorescence dyes to form bacterial targeting probes, which can selectively label bacteria in complex samples [60–62]. The combination of lipopolysaccharide-targeted polymyxin B and Cy3 dye can form a Gram-negative bacterial-specific probe [61]. The peptidoglycan-targeted vancomycin Cy3 conjugation probe could label Gram-positive bacteria after incubation with intestinal flora [62]. The most significant concern of those probes is the toxicity. Even low concentrations of antibiotic-based imaging probes could damage bacteria, resulting in drug resistance and host-microbiota disorder [63, 64].

Consequently, the metabolic labeling strategy is proposed to solve this problem. Metabolic-based mimic probes such as unnatural precursors or substrates could tag bacteria during proliferation or energy harvesting. And it has been applied to track gut microbiota colonization and spatial distribution [65]. The near-infrared sulfide quantum dots (PbS QD) coated with ribonuclease A (RNase-A) could be assembled with bacterial surface proteins [66]. The filamentous temperature-sensitive protein (FtsZ) inhibitor oxazolbenzamide is attached to a fluorophore that can mark *Staphylococcus aureus*, *Enterococcus faecalis*, *Streptococcus pneumoniae*, *Escherichia coli*, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa* [67]. But this strategy would fail if the bacterial metabolic pathways changed, which is likely to happen in gut microbiota.

Due to negative surface charges, different metal cation sterilants are used [68]. Researchers also utilize the contrast reagent labeled metal cations for bacteria imaging. The zinc(II)-coordinated compound is combined with the near-infrared fluorescence group, and the probe has high specificity for wounds infected by *Staphylococcus aureus* [69]. Combining antimicrobial peptide G3KL with fluorescent dyes can target Gram-negative bacteria and accumulate on the cell membrane [70]. AIE material TBP-1 could target the cell membrane of methicillin-resistant *Staphylococcus* with no drug resistance [71]. These probes have a simple chemical structures which are easy to synthesize, but they still face the same safety concern as antibiotic-based probes [72]. Positive charged dye MitoTracker Red is adopted in bacteria *in vitro* fluorescent imaging [73]. However, the cost is expensive due to the large amount needed for *in vivo* labeling by intragastric administration. On the other hand, some cation probes like cationic peptides could easily penetrate the mammalian cells, resulting in high background noise [74, 75].

In summary, despite deficiencies and shortcomings, fluorescence imaging technology is highly valued in probiotic quantification. The advantages of high sensitivity, accurate resolution, and low cost make it widely used in bacterial quantification and imaging. Better fluorescent probes and labeling strategies are eagerly needed to solve all the above concerns.

5. *In Vitro* and *In Vivo* Models

5.1. Bacterial Growth Inhibition Models. *Escherichia coli*, *Salmonella*, *Listeria*, *Clostridium difficile*, *Helicobacter pylori*, and *Candida albicans* are common pathogens that cause

gastrointestinal diseases. A large number of studies have used the traditional *in vitro* determination of the growth inhibition of probiotics on pathogenic bacteria to screen and assess the potential efficacy of probiotics (Figure 2). Abishad et al. investigated the effects of *Lactobacillus acidophilus* on a multi-drug-resistant enteroaggregative *Escherichia coli* (MDR-EAEC) strain, and they proved potential antibacterial and antibiofilm activity of green synthesized silver nanoparticles against MDR-EAEC strains with antioxidant properties [76]. Ruiz et al. used the synbiotic combination of *Bifidobacterium longum* subsp. *infantis* CECT7210 and oligosaccharides to examine the antimicrobial activity against *Escherichia coli*, *Cronobacter sakazakii*, *Listeria monocytogenes*, and *Clostridium difficile* in coculture experiments, and they found that the new symbiotic may be an effective supplement for infant health [77]. Cizeikiene and Jagelaviciute evaluated the antibacterial activities of twelve pathogenic strains belonging to *Staphylococcus aureus*, *Escherichia coli*, *Staphylococcus chromogenes*, and *Staphylococcus hyicus* species by performing agar diffusion assay and broth inhibition assay methods. The results demonstrated that *Lactobacillus acidophilus* DSM 20079, *Bifidobacterium pseudolongum* DSM 20099, and *Bifidobacterium animalis* DSM 20105 can serve as probiotic candidates [78].

Except for detecting the growth of pathogens, pathogenic genes or genes related to host infection were also utilized for screening probiotics. Wei et al. found that *Bifidobacterium longum* JDM301 not only played important roles in the growth inhibition against *C. difficile* but also directly promoted the degradation of clostridial toxin [79]. Ghadimi et al. evaluated the probiotic effects of *Bifidobacterium animalis* R101-8 by detecting the expression levels of key lipid metabolism genes, inflammation-related cytokines, and biomarkers, and they demonstrated that *B. animalis* R101-8 can improve biomarkers of metainflammation through the molecular/signaling mechanisms triggered by proinflammatory bacteria and lipids [80]. Additionally, with the rapid development of big data science and bioinformatics, some silicon models have also been developed for probiotic function evaluation. Mathematical models and genome scale metabolic models have been used for predicting and evaluating some bacterial probiotic functions [81, 82].

5.2. In Vitro Intestinal Microbiota Simulation Models. A more recent batch fermentation method simulating the distal colon could potentially be used for studying probiotic pathogen interactions. Models like SHIME, SIMGI, TIM-2, ECSIM, EnteroMix, and PolyFermS have been built for the human intestinal microbiota simulation [83]. Although most of these models were used for investigating the interactions between dietary functional factors or drugs and intestinal microbiota, the effect of probiotics, prebiotics, and synbiotics on the gut microbiota has been investigated by using these models. Duque et al. found that the probiotic, prebiotic, and synbiotic treatments resulted in a positive modulation of the gut microbiota and metabolic activity of children with autism spectrum disorder by using SHIME [84]. Marzorati et al. investigated the effects of MegaSporeBiotic™ (an oral, spore-based probiotic comprised of five Bacillus spp.) on gut microbiota activity and community composition using the SHIME, and they found that during treatment, *Akkermansia muciniphila*, *Bifidobacteria* spp., and Firmicutes increased while *Lactobacillus* spp. and Bacteroidetes decreased [85]. Due to the limited studies, the usability and feasibility of *in vitro* intestinal microbiota simulation models need to be further investigated.

5.3. Cell and Organoid Models. There are different models available that mimic the human intestinal epithelium and are thus available for studying probiotic and pathogen interactions in the gastrointestinal tract. Standard 2D models are comprised of culture plates as well as Transwell inserts, and newer 3D models like organoids, enteroids, and organ-on-a-chip have been built to assess probiotic-pathogen interactions [86]. Chen et al. found that the inhibition of *H. pylori* adhesion and the invasion of gastric epithelial cells and interleukin-8 production were significantly decreased by treatment with the *Lactobacillus* strains by using an *in vitro* cell-based model [87]. Wei et al. proved that *Bifidobacterium longum* JDM301 partially relieved damage to tissues caused by *C. difficile* and also decreased the number of *C. difficile* and toxin levels by using *in vitro* cell models [79]. Engevik et al. assessed the role of *Lactobacillus reuteri* in modulating the host immune system in an organoid-dendritic coculture and demonstrated that both *L. reuteri* secreted factors and its bacterial components are able to promote dendritic cell maturation [88]. In addition, some *ex vivo* models, like the Microbiota-human Intestine on chip (MihI-oC) [89], the Ussing chamber [90], and the human intestinal *in vitro* organ culture (IVOC) model [91], also have been developed. Efforts are under way to develop broader systems that connect multiple organotypic models to ultimately converge into a "body-on-a-chip" [92].

5.4. Animal Models. Animal models provide very controlled environments and enable the use of germ-free animals for studying the interactions between host and microbe as well as potential pathogens. In addition, animal models provide the possibility to collect samples from different parts of the gastrointestinal tract that is not possible within clinical trials. Although mice and rats are most frequently used, Caenorhabditis elegans, honey bees, Ciona robusta, fruit flies, and greater wax moths also have been developed for assessing probiotic-pathogen interactions [86]. Chen et al. indicated that colonization of *H. pylori* and induced stomach inflammation were alleviated by *Lactobacillus* strains [87]. Scalfaro et al. used *G. mellonella* to evaluate the antibacterial activity of *L. rhamnosus GG* and *Clostridium butyricum Miyairi* against three enteric pathogens causing infections, and these results suggested that *G. mellonella* larvae are a potentially useful *in vivo* model, which can complement *in vitro* assays to prescreen candidate probiotics [93]. Although genome editing technology has made rapid progress and many genome editing rats and mice have been established, there are still few cases of genome editing animals used in probiotic-related research. Hence, more studies on animal models are needed.

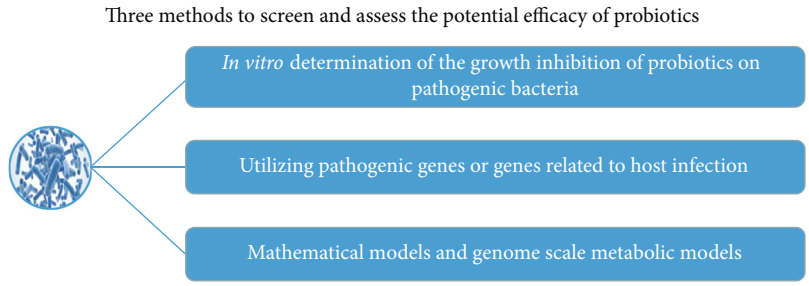

Figure 2: Three methods to screen and assess the potential efficacy of probiotics.

5.5. Human Clinical Experiments. Regardless of the fact that *in vitro* models and animal experiments have the advantages of simple operation, controllable experimental factors, and low research cost, reliable evidence of the impact of probiotics on human health still largely depends on human clinical experiments. Numerous studies have also further promoted the development and sales of probiotic products through human clinical trials. For example, *E. coli* includes a variety of strains, most of which are regarded as opportunistic pathogens. Human clinical trials are one of the important reasons why *Escherichia coli* Nissle 1917 is recognized as a probiotic and widely accepted and used [94]. Dronkers et al. performed the global analysis of clinical trials with probiotics, and they found that *L. rhamnosus GG* and *Bifidobacterium animalis ssp. lactis* BB12 are the most widely studied probiotic strains [95]. Although there exist several clinical trials to investigate the benefits of probiotics in gastrointestinal diseases, the results can be inconsistent and sometimes contrary, which may cause by many factors, including trail design, group size, group characteristics, and dosage. Hence, the design of human clinical experiments should consider probiotics, host population, and study design carefully.

6. Conclusions

Although probiotics can serve as therapeutic agents in treating gastrointestinal diseases, there are still several challenges, which may limit the effective applications. Understanding the existing challenges will make better use of probiotics in gastrointestinal diseases. Importantly, if probiotics cannot be delivered to the targeted place, the anticipated effect will not be achieved. Bacteriocins and bacteriocin-producing probiotics can maintain microecological balance and gut health, whereas troubles such as large-scale production and instability under the certain environments limit their further application. For probiotic quantification, fluorescence imaging technology has been concerned, and better fluorescent probes and labeling strategies are required to solve the existing drawbacks. Up to now, several *in vitro* and *in vivo* models have been developed to assess the potential efficacy of probiotic, including bacterial growth inhibition models, *in vitro* intestinal microbiota simulation models, cell and organoid models, animal models, and human clinical experiments. Nevertheless, the development of these models is still in the initial stage, and more efficient and proper models need to be established.

Conflicts of Interest

The authors declare no competing interests.

Authors' Contributions

Hang Xiao and William Wolfe developed the idea, and William Wolfe drafted the manuscript. Ze Xiang, Xi Yu, and Ping Li helped with the table and figures. Hao Chen, Mingfei Yao, Yiqiu Fei, and Yilun Huang revised the manuscript. Yeshi Yin developed the idea and drafted the outline. Hang Xiao organized, reviewed and finalized the manuscript. All authors contributed to the manuscript and approved for publication.

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References

[1] H. Shen, Z. Zhao, Z. Zhao, Y. Chen, and L. Zhang, "Native and engineered probiotics: promising agents against related systemic and intestinal diseases," *International Journal of Molecular Sciences*, vol. 23, no. 2, p. 594, 2022.

[2] M. Sanders, D. Merenstein, C. Merrifield, and R. Hutkins, "Probiotics for human use," *Nutrition bulletin*, vol. 43, no. 3, pp. 212–225, 2018.

[3] R. Guevarra and V. L. Barraquio, "Viable counts of lactic acid bacteria in Philippine commercial yogurts," *International Journal of Dairy Science & Processing*, vol. 2, no. 5, pp. 24–28, 2015.

[4] C. Picard, J. Fioramonti, A. Francois, T. Robinson, F. Neant, and C. Matuchansky, "Review article: bifidobacteria as probiotic agents – physiological effects and clinical benefits," *Alimentary Pharmacology & Therapeutics*, vol. 22, no. 6, pp. 495–512, 2005.

[5] R. Choudhary and R. Mahadevan, "Toward a systematic design of smart probiotics," *Current Opinion in Biotechnology*, vol. 64, pp. 199–209, 2020.

[6] S. Razavi, S. Janfaza, N. Tasnim, D. L. Gibson, and M. Hoorfar, "Microencapsulating polymers for probiotics delivery systems:

preparation, characterization, and applications," *Food Hydrocolloids*, vol. 120, no. 10, article 106882, 2021.

[7] C. González-Ferrero, J. M. Irache, and C. González-Navarro, "Soybean protein-based microparticles for oral delivery of probiotics with improved stability during storage and gut resistance," *Food Chemistry*, vol. 239, pp. 879–888, 2018.

[8] H. J. Chen, D. A. Lin, F. Liu et al., "Transdermal Delivery of Living and Biofunctional Probiotics through Dissolvable Microneedle Patches," *ACS Applied Bio Materials*, vol. 1, no. 2, pp. 374–381, 2018.

[9] A. Gani, A. Shah, M. Ahmad, B. A. Ashwar, and F. A. Masoodi, "β-d-glucan as an enteric delivery vehicle for probiotics," *International Journal of Biological Macromolecules*, vol. 106, pp. 864–869, 2018.

[10] K. Kailasapathy, J. Versalovic, and M. Wilson, "Formulation, Administration, and Delivery of Probiotics," in *Therapeutic Microbiology: Probiotics and Related Strategies*, Wiley, 2014.

[11] A. C. Anselmo, K. J. Mchugh, J. Webster, R. Langer, and A. Jaklenec, "Biomaterials: layer-by-layer encapsulation of probiotics for delivery to the microbiome (Adv. Mater. 43/2016)," *Advanced Materials*, vol. 28, no. 43, pp. 9442–9442, 2016.

[12] C. G. Olnood, S. S. M. Beski, P. A. Iji, and M. Choct, "Delivery Routes for Probiotics: Effects on Broiler Performance, Intestinal Morphology and Gut Microflora," vol. 1, Tech. Rep. 3, Animal Nutrition, 2015.

[13] R. Gheorghita, L. Anchidin-Norocel, R. Filip, M. Dimian, and M. Covasa, "Applications of biopolymers for drugs and probiotics delivery," *Polymers*, vol. 13, no. 16, p. 2729, 2021.

[14] J. Kim, S. P. Hlaing, J. Lee, A. Saparbayeva, and J. W. Yoo, "Exfoliated bentonite/alginate nanocomposite hydrogel enhances intestinal delivery of probiotics by resistance to gastric pH and on-demand disintegration," *Carbohydrate Polymers*, vol. 272, no. 42, article 118462, 2021.

[15] L. I. Li-Juan and S. F. School, "Effects of dietary chitosan and probiotics on growth and non-specific immunity of Pelteobagrus fulvidraco," *Journal of Anhui Agricultural Ences*, vol. 26, no. 6, pp. 2614–2619, 2013.

[16] L. P. Ta, E. Bujna, S. Kun, D. Charalampopoulos, and V. V. Khutoryanskiy, "Electrosprayed mucoadhesive alginate-chitosan microcapsules for gastrointestinal delivery of probiotics," *International Journal of Pharmaceutics*, vol. 597, article 120342, 2021.

[17] C. C. Dodoo, J. Wang, A. W. Basit, P. Stapleton, and S. Gaisford, "Targeted delivery of probiotics to enhance gastrointestinal stability and intestinal colonisation," *International Journal of Pharmaceutics*, vol. 530, no. 1-2, pp. 224–229, 2017.

[18] C. Iyer, "Studies on Co-Encapsulation of Probiotics and Prebiotics and Its Efficacy in Survival, Delivery, Release and Immunomodulatory Activity in the Host Intestine, [Ph.D. thesis]," University of Western Sydney, Australia, 2005.

[19] P. Devani, A. Ruparelia, V. Garcia-Larsen et al., "Optimal Mode of Delivery for Using Probiotics or Prebiotics to Prevent Eczema: A Systematic Review and Meta-Analysis," *Clinical and Experimental Allergy*, vol. 47, no. 12, p. 1700, 2017.

[20] N. Kevin, A. Nancy, R. Patricia et al., "High-efficiency delivery of CRISPR-Cas9 by engineered probiotics enables precise microbiome editing," *Molecular Systems Biology*, vol. 17, no. 10, 2021.

[21] P. D. Cotter, C. Hill, and R. P. Ross, "Bacteriocins: developing innate immunity for food," *Nature Reviews Microbiology*, vol. 3, no. 10, pp. 777–788, 2005.

[22] O. Gillor, A. Etzion, and M. A. Riley, "The dual role of bacteriocins as anti- and probiotics," *Applied Microbiology and Biotechnology*, vol. 81, no. 4, pp. 591–606, 2008.

[23] Ö. C. O. Umu, C. Bäuerl, M. Oostindjer et al., "The potential of class II bacteriocins to modify gut microbiota to improve host health," *PLoS One*, vol. 11, no. 10, article e0164036, 2016.

[24] Y. Cui, L. Luo, X. Wang et al., "Mining, heterologous expression, purification, antibactericidal mechanism, and application of bacteriocins: a review," *Comprehensive Reviews in Food Science and Food Safety*, vol. 20, no. 1, pp. 863–899, 2021.

[25] E. M. Johnson, D. Y.-G. Jung, D. Y.-Y. Jin, D. R. Jayabalan, D. S. H. Yang, and J. W. Suh, "Bacteriocins as food preservatives: challenges and emerging horizons," *Critical Reviews in Food Science and Nutrition*, vol. 58, no. 16, pp. 2743–2767, 2018.

[26] R. Kumariya, A. K. Garsa, Y. S. Rajput, S. K. Sood, N. Akhtar, and S. Patel, "Bacteriocins: classification, synthesis, mechanism of action and resistance development in food spoilage causing bacteria," *Microbial Pathogenesis*, vol. 128, pp. 171–177, 2019.

[27] P. D. Cotter, R. P. Ross, and C. Hill, "Bacteriocins – a viable alternative to antibiotics?," *Nature Reviews Microbiology*, vol. 11, no. 2, pp. 95–105, 2013.

[28] S. Heu, J. Oh, Y. Kang et al., "gly gene cloning and expression and purification of glycinecin A, a bacteriocin produced by Xanthomonas campestris pv. glycines 8ra," *Applied and Environmental Microbiology*, vol. 67, no. 9, pp. 4105–4110, 2001.

[29] S. D. Todorov, B. D. G. de Melo Franco, and J. R. Tagg, "Bacteriocins of gram-positive bacteria having activity spectra extending beyond closely-related species," *Beneficial Microbes*, vol. 10, no. 3, pp. 315–328, 2019.

[30] G. G. F. Newton, E. P. Abraham, and N. J. Berridge, "Sulphur-containing amino-acids of nisin," *Nature*, vol. 171, no. 4353, p. 606, 1953.

[31] D. D. Heeney, Z. Zhai, Z. Bendiks et al., "*Lactobacillus plantarum* bacteriocin is associated with intestinal and systemic improvements in diet-induced obese mice and maintains epithelial barrier integrity *in vitro*," *Gut Microbes*, vol. 10, no. 3, pp. 382–397, 2019.

[32] T. M. Kuniyoshi, P. M. O'Connor, E. Lawton et al., "An oxidation resistant pediocin PA-1 derivative and penocin A display effective anti-listeria activity in a model human gut environment," *Gut Microbes*, vol. 14, no. 1, 2022.

[33] C. Madera, P. García, A. Rodríguez, J. E. Suárez, and B. Martínez, "Prophage induction in *Lactococcus lactis* by the bacteriocin Lactococcin 972," *International Journal of Food Microbiology*, vol. 129, no. 1, pp. 99–102, 2009.

[34] G. C. Upreti and R. D. Hinsdill, "Production and mode of action of lactocin 27: bacteriocin from a homofermentative Lactobacillus," *Antimicrobial Agents and Chemotherapy*, vol. 7, no. 2, pp. 139–145, 1975.

[35] R. Kemperman, A. Kuipers, H. Karsens, A. Nauta, O. Kuipers, and J. Kok, "Identification and characterization of two novel clostridial bacteriocins, circularin A and closticin 574," *Applied and Environmental Microbiology*, vol. 69, no. 3, pp. 1589–1597, 2003.

[36] F. Drissi, S. Buffet, D. Raoult, and V. Merhej, "Common occurrence of antibacterial agents in human intestinal microbiota," *Frontiers in Microbiology*, vol. 6, p. 441, 2015.

[37] J. W. Hegarty, C. M. Guinane, R. P. Ross, C. Hill, and P. D. Cotter, "Bacteriocin production: a relatively unharnessed probiotic trait?," *F1000Research*, vol. 5, p. 2587, 2016.

[38] M. Millette, G. Cornut, C. Dupont, F. Shareck, D. Archambault, and M. Lacroix, "Capacity of human nisin- and pediocin-producing lactic acid bacteria to reduce intestinal colonization by vancomycin-resistant enterococci," *Applied and Environmental Microbiology*, vol. 74, no. 7, pp. 1997–2003, 2008.

[39] E. F. Murphy, P. D. Cotter, A. Hogan et al., "Divergent metabolic outcomes arising from targeted manipulation of the gut microbiota in diet-induced obesity," *Gut*, vol. 62, no. 2, pp. 220–226, 2013.

[40] E. Pessione, "Lactic acid bacteria contribution to gut microbiota complexity: lights and shadows," *Frontiers in Cellular and Infection Microbiology*, vol. 2, 2012.

[41] T. A. Fuchs, U. Abed, C. Goosmann et al., "Novel cell death program leads to neutrophil extracellular traps," *Journal of Cell Biology*, vol. 176, no. 2, pp. 231–241, 2007.

[42] T. Wang, J. Fu, X. Xiao et al., "CBP22, a Novel Bacteriocin Isolated from *Clostridium butyricum* ZJU-F1, Protects against LPS-Induced Intestinal Injury through Maintaining the Tight Junction Complex," *Mediators of Inflammation*, vol. 2021, Article ID 8032125, 12 pages, 2021.

[43] D. Begde, S. Bundale, P. Mashitha, J. Rudra, N. Nashikkar, and A. Upadhyay, "Immunomodulatory efficacy of nisin–a bacterial lantibiotic peptide," *Journal of Peptide Science*, vol. 17, no. 6, pp. 438–444, 2011.

[44] X. Yin, D. Heeney, Y. Srisengfa, B. Golomb, S. Griffey, and M. Marco, "Bacteriocin biosynthesis contributes to the anti-inflammatory capacities of probiotic Lactobacillus plantarum," *Beneficial Microbes*, vol. 9, no. 2, pp. 333–344, 2018.

[45] H. N. Bell, R. J. Rebernick, J. Goyert et al., "Reuterin in the healthy gut microbiome suppresses colorectal cancer growth through altering redox balance," *Cancer Cell*, vol. 40, no. 2, pp. 185–200.e6, 2022.

[46] Z. Norouzi, A. Salimi, R. Halabian, and H. Fahimi, "Nisin, a potent bacteriocin and anti-bacterial peptide, attenuates expression of metastatic genes in colorectal cancer cell lines," *Microbial Pathogenesis*, vol. 123, pp. 183–189, 2018.

[47] A. De Giani, F. Bovio, M. Forcella, P. Fusi, G. Sello, and P. Di Gennaro, "Identification of a bacteriocin-like compound from Lactobacillus plantarum with antimicrobial activity and effects on normal and cancerogenic human intestinal cells," *AMB Express*, vol. 9, no. 1, 2019.

[48] P. Baindara, S. Korpole, and V. Grover, "Bacteriocins: perspective for the development of novel anticancer drugs," *Applied Microbiology and Biotechnology*, vol. 102, no. 24, pp. 10393–10408, 2018.

[49] S. Kaur and S. Kaur, "Bacteriocins as potential anticancer agents," *Frontiers in Pharmacology*, vol. 6, 2015.

[50] J. W. Goyert, H. N. Bell, and Y. M. Shah, "PCL22-188: reuterin in the healthy gut microbiome suppresses colorectal cancer growth through altering redox balance," *Journal of the National Comprehensive Cancer Network*, vol. 20, no. 3.5, pp. PCL22–PC188, 2022.

[51] E. Meade, M. A. Slattery, and M. Garvey, "Bacteriocins, potent antimicrobial peptides and the fight against multi drug resistant species: resistance is futile?," *Antibiotics*, vol. 9, no. 1, p. 32, 2020.

[52] H. Mathur, D. Field, M. C. Rea, P. D. Cotter, C. Hill, and R. P. Ross, "Fighting biofilms with lantibiotics and other groups of bacteriocins," *npj Biofilms and Microbiomes*, vol. 4, no. 1, p. 9, 2018.

[53] J. Qin, M. H. I. T. Consortium, R. Li et al., "A human gut microbial gene catalogue established by metagenomic sequencing," *Nature*, vol. 464, no. 7285, pp. 59–65, 2010.

[54] G. P. Donaldson, S. M. Lee, and S. K. Mazmanian, "Gut biogeography of the bacterial microbiota," *Nature Reviews. Microbiology*, vol. 14, no. 1, pp. 20–32, 2016.

[55] C. Tropini, K. A. Earle, K. C. Huang, and J. L. Sonnenburg, "The gut microbiome: connecting spatial organization to function," *Cell Host & Microbe*, vol. 21, no. 4, pp. 433–442, 2017.

[56] B. Lim, M. Zimmermann, N. A. Barry, and A. L. Goodman, "Engineered regulatory systems modulate gene expression of human commensals in the gut," *Cell*, vol. 169, no. 3, pp. 547–558.e15, 2017.

[57] W. R. Whitaker, E. S. Shepherd, and J. L. Sonnenburg, "Tunable expression tools enable single-cell strain distinction in the gut microbiome," *Cell*, vol. 169, no. 3, pp. 538–546.e12, 2017.

[58] M. Barbier, J. Bevere, and F. H. Damron, "In vivo bacterial imaging using bioluminescence," in *Methods In Molecular Biology*, pp. 87–97, Springer, (Clifton, N.J), 2018.

[59] M. E. V. Johansson and G. C. Hansson, "Preservation of mucus in histological sections, immunostaining of mucins in fixed tissue, and localization of bacteria with FISH," in *Methods in Molecular Biology*, vol. 842, pp. 229–235, Clifton, N.J, 2012.

[60] Y. Imai, K. J. Meyer, A. Iinishi et al., "A new antibiotic selectively kills gram-negative pathogens," *Nature*, vol. 576, no. 7787, pp. 459–464, 2019.

[61] W. Wang and X. Chen, "Antibiotics-based fluorescent probes for selective labeling of gram-negative and gram-positive bacteria in living microbiotas," *Science China Chemistry*, vol. 61, no. 7, pp. 792–796, 2018.

[62] W. Wang, Y. Zhu, and X. Chen, "Selective imaging of gram-negative and gram-positive microbiotas in the mouse gut," *Biochemistry*, vol. 56, no. 30, pp. 3889–3893, 2017.

[63] F. Faber, L. Tran, M. X. Byndloss et al., "Host-mediated sugar oxidation promotes post-antibiotic pathogen expansion," *Nature*, vol. 534, no. 7609, pp. 697–699, 2016.

[64] F. Rivera-Chávez, L. F. Zhang, F. Faber et al., "Depletion of Butyrate-Producing *Clostridia* from the Gut Microbiota Drives an Aerobic Luminal Expansion of *Salmonella*," *Cell Host & Microbe*, vol. 19, no. 4, pp. 443–454, 2016.

[65] W. Wang, L. Lin, Y. Du et al., "Assessing the viability of transplanted gut microbiota by sequential tagging with D-amino acid-based metabolic probes," *Nature Communications*, vol. 10, no. 1, 2019.

[66] J. Chen, S. Feng, M. Chen et al., "In vivo dynamic monitoring of bacterial infection by NIR-II fluorescence imaging," *Small*, vol. 16, no. 34, article 2002054, 2020.

[67] E. Ferrer-González, J. Fujita, T. Yoshizawa et al., "Structure-guided design of a fluorescent probe for the visualization of FtsZ in clinically important gram-positive and gram-negative bacterial pathogens," *Scientific Reports*, vol. 9, no. 1, 2019.

[68] L. Pasquina-Lemonche, J. Burns, R. D. Turner et al., "The architecture of the gram-positive bacterial cell wall," *Nature*, vol. 582, no. 7811, pp. 294–297, 2020.

[69] W. M. Leevy, S. T. Gammon, J. R. Johnson et al., "Noninvasive optical imaging of *staphylococcus aureus* bacterial infection in living mice using a Bis-dipicolylamine-zinc (II)

affinity group conjugated to a near-infrared fluorophore," *Bioconjugate Chemistry*, vol. 19, no. 3, pp. 686–692, 2008.

[70] B.-H. Gan, T. N. Siriwardena, S. Javor, T. Darbre, and J.-L. Reymond, "Fluorescence imaging of bacterial killing by antimicrobial peptide dendrimer G3KL," *ACS infectious diseases*, vol. 5, no. 12, pp. 2164–2173, 2019.

[71] Y. Li, F. Liu, J. Zhang et al., "Efficient killing of multidrug-resistant internalized bacteria by AIEgens in vivo," *Advanced Science*, vol. 8, no. 9, article 2001750, 2021.

[72] F. Hu, G. Qi, D. Mao et al., "Visualization and in situ ablation of intracellular bacterial pathogens through metabolic labeling," *Angewandte Chemie International Edition*, vol. 59, no. 24, pp. 9288–9292, 2020.

[73] I. Maslov, A. Bogorodskiy, A. Mishin et al., "Efficient non-cytotoxic fluorescent staining of halophiles," *Scientific Reports*, vol. 8, no. 1, 2018.

[74] K. E. Bullok, S. T. Gammon, S. Violini et al., "Permeation peptide conjugates for in vivo molecular imaging applications," *Molecular Imaging*, vol. 5, no. 1, pp. 1–15, 2006.

[75] N. Nekhotiaeva, A. Elmquist, G. K. Rajarao, M. Hallbrink, U. Langel, and L. Good, "Cell entry and antimicrobial properties of eukaryotic cell-penetrating peptides," *The FASEB Journal*, vol. 18, no. 2, pp. 1–15, 2004.

[76] P. Abishad, J. Vergis, V. Unni et al., "Green synthesized silver nanoparticles using Lactobacillus acidophilus as an antioxidant, antimicrobial, and antibiofilm agent against multidrug resistant enteroaggregative Escherichia coli," *Probiotics and Antimicrobial Proteins*, vol. 14, no. 5, pp. 904–914, 2022.

[77] L. Ruiz, A. B. Flórez, B. Sánchez et al., "Bifidobacterium longum subsp. infantis CECT7210 (B. infantis IM-1®) displays in vitro activity against some intestinal pathogens," *Nutrients*, vol. 12, no. 11, p. 3259, 2020.

[78] D. Cizeikiene and J. Jagelaviciute, "Investigation of antibacterial activity and probiotic properties of strains belonging to Lactobacillus and Bifidobacterium genera for their potential application in functional food and feed products," *Probiotics and Antimicrobial Proteins*, vol. 13, no. 5, pp. 1387–1403, 2021.

[79] Y. Wei, F. Yang, Q. Wu et al., "Protective effects of bifidobacterial strains against toxigenic Clostridium difficile," *Frontiers in Microbiology*, vol. 9, 2018.

[80] D. Ghadimi, A. Nielsen, M. F. Hassan et al., "Modulation of proinflammatory bacteria-and lipid-coupled intracellular signaling pathways in a Transwell triple co-culture model by commensal Bifidobacterium animalis R101-8," *Anti-Inflammatory & Anti-Allergy Agents in Medicinal Chemistry*, vol. 20, no. 2, pp. 161–181, 2021.

[81] J. C. Arciero, G. B. Ermentrout, J. S. Upperman, Y. Vodovotz, and J. E. Rubin, "Using a mathematical model to analyze the role of probiotics and inflammation in necrotizing enterocolitis," *PLoS One*, vol. 5, no. 4, article e10066, 2010.

[82] Y.-M. Choi, Y. Q. Lee, H.-S. Song, and D.-Y. Lee, "Genome scale metabolic models and analysis for evaluating probiotic potentials," *Biochemical Society Transactions*, vol. 48, no. 4, pp. 1309–1321, 2020.

[83] A. Gościniak, P. Eder, J. Walkowiak, and J. Cielecka-Piontek, "Artificial gastrointestinal models for nutraceuticals research—achievements and challenges: a practical review," *Nutrients*, vol. 14, no. 13, p. 2560, 2022.

[84] A. L. R. F. Duque, F. M. Demarqui, M. M. Santoni et al., "Effect of probiotic, prebiotic, and synbiotic on the gut microbiota of autistic children using an *in vitro* gut microbiome model," *Food Research International*, vol. 149, article 110657, 2021.

[85] M. Marzorati, P. Van den Abbeele, S. Bubeck, T. Bayne, K. Krishnan, and A. Young, "Treatment with a spore-based probiotic containing five strains of *Bacillus* induced changes in the metabolic activity and community composition of the gut microbiota in a SHIME ® model of the human gastrointestinal system," *Food Research International*, vol. 149, article 110676, 2021.

[86] M. Anjum, A. Laitila, A. C. Ouwehand, and S. D. Forssten, "Current perspectives on gastrointestinal models to assess probiotic-pathogen interactions," *Frontiers in Microbiology*, vol. 13, 2022.

[87] Y.-H. Chen, W.-H. Tsai, H.-Y. Wu et al., "Probiotic Lactobacillus spp. act against Helicobacter pylori-induced inflammation," *Journal of Clinical Medicine*, vol. 8, no. 1, p. 90, 2019.

[88] M. A. Engevik, W. Ruan, M. Esparza et al., "Immunomodulation of dendritic cells by *Lactobacillus reuteri* surface components and metabolites," *Physiological Reports*, vol. 9, no. 2, article e14719, 2021.

[89] V. De Gregorio, C. Sgambato, F. Urciuolo, R. Vecchione, P. A. Netti, and G. Imparato, "Immunoresponsive microbiota-gut-on-chip reproduces barrier dysfunction, stromal reshaping and probiotics translocation under inflammation," *Biomaterials*, vol. 286, article 121573, 2022.

[90] C. Guan, Y. Yang, D. Tian et al., "Evaluation of an Ussing chamber system equipped with rat intestinal tissues to predict intestinal absorption and metabolism in humans," *European Journal of Drug Metabolism and Pharmacokinetics*, vol. 47, no. 5, pp. 639–652, 2022.

[91] A. Haque, F. Bowe, R. Fitzhenry et al., "Early interactions of Salmonella enterica serovar Typhimurium with human small intestinal epithelial explants," *Gut*, vol. 53, no. 10, pp. 1424–1430, 2004.

[92] U. Marx, E. Accastelli, R. David et al., "An individual patient's "body" on chips—how organismoid theory can translate into your personal precision therapy approach," *Frontiers in Medicine*, vol. 8, 2021.

[93] C. Scalfaro, A. Iacobino, C. Nardis, and G. Franciosa, "Galleria mellonella as an in vivo model for assessing the protective activity of probiotics against gastrointestinal bacterial pathogens," *FEMS Microbiology Letters*, vol. 364, no. 7, 2017.

[94] Z. Zhao, S. Xu, W. Zhang, D. Wu, and G. Yang, "Probiotic Escherichia coli NISSLE 1917 for Inflammatory Bowel Disease Applications," *Food & Function*, vol. 13, no. 11, pp. 5914–5924, 2022.

[95] T. M. Dronkers, A. C. Ouwehand, and G. T. Rijkers, "Global analysis of clinical trials with probiotics," *Heliyon*, vol. 6, no. 7, article e04467, 2020.

[96] L. Beaulieu, *Production, Purification et Caracterisation de la Pediocine PA-1 Naturelle et de ses Formes Recombinantes: Contribution a la Mise en Evidence d'une Nouvelle Activite Biologique (French and English text), [Ph.D. thesis]*, Universite Laval, Canada, 2004.

[97] H. Sareh Sadat, H. Bahareh, F. Ebrahim, and G. Hossein, "Increased expression of caspase genes in colorectal cancer cell line by nisin," *Archives of Clinical Infectious Diseases*, vol. 15, no. 2, 2020.

[98] A. Shiva, G. Marzieh, and H. Hamideh Mahmoodzadeh, "The apoptotic impact of nisin as a potent bacteriocin on the colon

cancer cells," *Microbial Pathogenesis*, vol. 111, pp. 193–197, 2017.

[99] S. Maher and S. McClean, "Investigation of the cytotoxicity of eukaryotic and prokaryotic antimicrobial peptides in intestinal epithelial cells in vitro," *Biochemical Pharmacology*, vol. 71, no. 9, pp. 1289–1298, 2006.

[100] L. M. John, "Managing potentially resectable metastatic colon cancer," *Gastrointestinal Cancer Research: GCR*, vol. 2, Supplement 4, pp. S23–S26, 2008.

[101] N. J. Roberts, L. Zhang, F. Janku et al., "Intratumoral injection of *Clostridium novyi*-NT spores induces antitumor responses," *Science Translational Medicine*, vol. 6, no. 249, article 249ra111, 2014.

[102] C. M. Guinane, E. M. Lawton, P. M. O'Connor et al., "The bacteriocin bactofencin A subtly modulates gut microbial populations," *Anaerobe*, vol. 40, pp. 41–49, 2016.

[103] F. Taghizad, H. R. Kazerani, H. Dehghani, A. Asoodeh, and D. Yaghubi, "A novel approach towards obesity: the use of a bacterial product, gassericin A, in 3T3-L1 cells," *Obesity Research & Clinical Practice*, vol. 15, no. 5, pp. 499–505, 2021.