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Challenges and Opportunities in the Industrial Application of Probiotics

Sahar Hemati[1], Hadis Farokhdel[2], Hadiseh Masoudi[2], Mehran Mahooti[3*], Davood Zare[3*]

[1] Department of Biology, Science and Research Branch, Islamic Azad University, Tehran, Iran.
[2] Department of biology, central Tehran branch, Islamic Azad University, Tehran, Iran
[3] Department of Biotechnology, Iranian Research Organization for Science and Technology, P. O. Box 3353-5111, Tehran, Iran

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***corresponding authors**
Mehran Mahooti:
Mehran.mahooti@gmail.com

Davood Zare,: zare@irost.ir ; davoodz@yahoo.com
Tel.: 98 21 82884383, Fax: 98 21 82884931,

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ABSTRACT

Background and Objective: The growing understanding of the relationship between nutrition, the gut microbiota, and human health has promoted the development of functional foods containing probiotics. However, maintaining the viability, stability, and biological activity of probiotics from strain selection to consumption remains a major challenge in the development and industrial production of these products. This review aimed to provide an integrated overview of probiotic strain selection, fermentation processes, and emerging strategies to improve probiotic viability, stability, and scalability in food applications, while highlighting key technological and industrial challenges.

Materials and Methods: This review was conducted through a systematic literature search focusing on probiotics, fermentation processes, and their applications in the food industry. Relevant scientific literature was retrieved from PubMed, Scopus, Google Scholar, and Nature. The search strategy was based on the following keywords: Probiotics, Functional Foods, Lactic Acid Bacteria, Fermentation, and Postbiotics. The retrieved studies were evaluated based on their relevance to the scope and objectives of this review, with particular emphasis on probiotic applications, fermentation processes, microbial viability and stability, and industrial scalability.

Results and Conclusion: Strain-specific characteristics, gastrointestinal tolerance, safety, adhesion capacity, resistance to environmental stresses, and the ability to survive processing and storage are among the key criteria for selecting suitable probiotic strains. On the other hand, fermentation, by modifying the nutritional, sensory, and functional properties of the food matrix, represents a critical step in the production of probiotic products. Nevertheless, factors such as processing conditions, oxygen exposure, moisture, temperature, and pH can significantly affect microbial viability. Optimization of fermentation processes, stress adaptation, metabolic engineering, and microencapsulation technologies are among the approaches that can improve probiotic resistance, stability, and scalability for industrial production. In this review article, the criteria influencing strain selection, the importance of fermentation, the factors determining probiotic viability and stability, and emerging strategies for improving their production and industrial applications are discussed, with the aim of promoting the sustainable development of functional foods.

What is "already known":	• Probiotics and bioactive compounds play important role in functional foods and gut health. • Probiotic viability and functionality are affected by fermentation, processing, storage, and gastrointestinal conditions.
What this article adds:	• The key factors influencing probiotic selection, production, stability, and functionality. • Strategies, including fermentation optimization, stress adaptation, and microencapsulation, to enhance probiotic viability. • Industrial challenges and future prospects for the development of stable and sustainable probiotic-based functional foods.

1. INTRODUCTION

With increasing attention to the relationship between nutrition, the gut microbiota, and human health, the application of probiotics and food bioactive compounds in the development of functional foods has expanded. Probiotics (live microorganisms that, when administered in adequate amounts, confer health benefits to the host) are incorporated into a wide range of dairy and non-dairy products, beverages, cereal-based foods, and other food matrices. Despite considerable advances, the precise mechanisms by which probiotics and bioactive compounds influence the gut microbiota and metabolism remain incompletely understood and are affected by factors such as diet, genetics, and the environment [1]. Fermentation is one of the key processes in the production of probiotic products and can influence product quality, sensory characteristics, nutritional value, and stability. During this process, strain characteristics, food matrix composition, and processing conditions interactively affect the growth, activity, and viability of microorganisms. Lactic acid bacteria (LAB) are among the most important microorganisms used in fermentation and are widely employed in the production of a broad range of food products. However, probiotics are exposed to various environmental stresses during production, processing, and storage, which may reduce their viability and functionality. Therefore, the selection of appropriate strains and the application of effective strategies to enhance stress tolerance and maintain probiotic stability are of great importance. Understanding the factors affecting the growth, resistance, and survival of probiotics can contribute to the development of more stable probiotic products with improved functionality [2]. Despite the high potential of probiotics for the development of functional foods, maintaining their viability and activity from production to consumption remains a major challenge in their industrial application. Probiotics are exposed to various factors during processing, packaging, storage, and gastrointestinal transit that can reduce their survival. The severity of these effects depends on the characteristics of the strain, the food matrix, and the processing conditions. Therefore, the selection of resistant and well-adapted strains, control of production and storage conditions, and the use of protective strategies are of great importance. Among these approaches, microencapsulation can provide a protective environment that enhances the stability and survival of probiotics under adverse conditions. Overall, optimizing processing conditions and employing protective strategies are essential for maintaining an adequate number of viable cells and preserving the biological activity of probiotics until consumption [3, 4]. The increasing demand for sustainable production has drawn attention to the use of microorganisms as cell factories. However, limitations in strain performance and production processes have posed challenges to the commercialization of microbial products. Systems metabolic engineering, through approaches such as host and pathway selection, metabolic pathway reconstruction, enhanced tolerance, optimization of metabolic flux and fermentation, integration of downstream processing, and scale-up, can improve strain and process performance and facilitate the development of industrial-scale production [5]. The development of probiotic-based functional foods is a multistage process that begins with the selection of suitable strains and continues through fermentation, biomass production, processing, storage, and product consumption. The success of this process depends on the interplay among strain characteristics, resistance to environmental stresses, fermentation conditions, food matrix properties, and processing and stabilization methods. Therefore, a comprehensive understanding of the factors affecting

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the viability and functionality of probiotics, along with the application of strategies such as fermentation process optimization, selection of stress-resistant strains, stress adaptation, and protective technologies, is essential for developing stable and reliable products. Accordingly, an integrated evaluation of the probiotic production pathway, from strain selection to the production and commercialization of the final product, can help identify existing challenges and facilitate the development of effective approaches to improve the stability, efficiency, and industrial applicability of probiotic-based functional foods [6]. The factors affecting the production, stability, and functionality of probiotics are presented in Table 1. In this review article, the criteria for selecting suitable strains for industrial applications, the role of fermentation processes in maintaining probiotic viability and functionality, the factors affecting their stability during processing and storage, and emerging strategies to improve the production, scalability, and stability of probiotic products are examined. In addition, the challenges associated with the transition of probiotics from the production stage to the final food product, as well as future opportunities for developing efficient and sustainable processes, are discussed.

Table 1. Factors influencing the selection, production, stability, and functionality of probiotics

Category	Criteria	Importance for probiotic products	References
Strain selection	Gastrointestinal survival, adhesion to intestinal epithelial cells	Essential for maintaining probiotic functionality and enabling the strain to exert beneficial effects in the gastrointestinal tract	[7–9, 12]
Gastrointestinal tolerance	Resistance to gastric acid, bile salts, and gastrointestinal conditions	Supports survival and delivery of viable probiotic cells to the intestine	[9, 14, 15]
Fermentation	Strain characteristics, food matrix composition, fermentation conditions	Influences probiotic growth, viability, product quality, nutritional properties, and functionality	[2, 16, 19, 23, 25]
Functional metabolites	γ-Aminobutyric acid, exopolysaccharides, conjugated linoleic acid, bacteriocins, and antimicrobial metabolites	May contribute to the nutritional, functional, antimicrobial, and health-promoting properties of probiotic foods	[13, 32, 48, 49]

2. MATERIALS AND METHODS

This review study was conducted through a literature search of electronic databases, including PubMed, Scopus, Google Scholar, and Nature, to identify relevant scientific publications related to probiotics and their applications in the food industry. The search strategy was developed using a combination of the following keywords: probiotics, functional foods, lactic acid bacteria, fermentation, and postbiotics. These keywords were selected to cover the main aspects of the review, including the role of beneficial microorganisms in food production, their incorporation into functional foods, fermentation processes, and the potential applications of postbiotics. Initially, the titles and abstracts of the retrieved publications were screened to identify studies relevant to the objectives of this review. Articles considered potentially relevant were then evaluated through full text examination. The selection process focused on studies addressing probiotic characteristics and applications, the role of lactic acid bacteria in fermentation, factors influencing microbial viability and stability, and the development of functional food products. Particular attention was also given to research discussing technological challenges, production processes, and the potential industrial applications of probiotics and postbiotics. Finally, the selected literature was assessed based on its

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scientific relevance, quality, and contribution to understanding current developments, challenges, and future perspectives in this field.

3. PROBIOTICS AND CRITERIA FOR SELECTING SUITABLE STRAINS FOR INDUSTRIAL APPLICATIONS

The health benefits of probiotics have been demonstrated in numerous scientific studies. This topic has received extensive media coverage, leading to the market launch of a wide range of probiotic supplements [7]. The key criteria studied for evaluating the suitability of probiotics regarding their nutritional effects on human health include: hemolytic activity; survival of microbial cultures in the gastrointestinal tract; their metabolic activity at the site of colonization; strain-host interaction; technological stability during storage; and biofilm-forming ability for effective intestinal colonization [8]. For probiotic strains to function effectively, they must possess specific beneficial characteristics, including tolerance to gastrointestinal conditions, adhesion to epithelial cells, cholesterol-binding ability, bile salt hydrolysis, safety regarding pathogenic genes, non-hemolytic activity, antibiotic susceptibility, antibacterial properties, and viability during fermentation and storage processes [9]. Probiotic bacteria must maintain high levels of viability during processing. During storage, transport, and specifically consumption, they are exposed to oxygen, moisture, heat, strong acids, and bile; to withstand these adverse conditions, various dosage forms such as capsules, tablets, powders, and liquids are employed [10]. Probiotic strains intended for human use must be free of pathogenic characteristics, with hemolytic activity serving as a key indicator of their potential pathogenicity. Gamma-hemolytic strains exhibit no hemolytic activity and are relatively safe [11]. From a microbiological perspective, probiotics must survive within the gastrointestinal tract's microbial ecosystem, and their consumption should ideally not cause adverse side effects such as bloating or impaired intestinal transit. To enhance their survival in the gut, probiotics must be capable of adhering to the mucosal surface [12]. For probiotics to function effectively in the intestine, following successful microbial adhesion to the intestinal lining, they can synthesize a wide range of antimicrobial compounds and bioactive metabolites, thereby aiding host defence through direct antagonistic activity against pathogenic microorganisms [13]. During digestion, probiotics first encounter the stomach environment. Gastric acid (with a pH of 2) is a highly acidic digestive fluid that affects probiotic survival; encapsulation protects the probiotics and ensures their survival as they pass through the stomach to the intestine, where they can effectively exert their beneficial effects [14, 15]. One of the fundamental aspects of safety assessment for any probiotic strain is the determination of its complete genome sequence. This enables the assignment of the strain to a specific taxonomic group, facilitates the examination of known risks associated with that species, and allows for precise identification at the strain level a capability that is significant for tracking the strain throughout the production process and investigating the etiology of potential infections [16]. One of the key issues regarding the safety of modern probiotics is the assessment of their potential for antibiotic resistance, which has become a major priority; this is because there is a risk of resistance genes being transferred from probiotics to pathogenic gut bacteria via horizontal gene transfer, a phenomenon that could pose a challenge to public health [13]. Future research should focus on the strain-specific safety assessment of probiotics through genomic analyses to identify virulence factors and transferable antimicrobial resistance genes. Since probiotic safety depends on the strain, host, and administered dose, each strain should be evalua-

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ted individually using combined in silico, in vitro, and in vivo approaches. Comprehensive safety assessments and continuous monitoring during production and storage are essential to ensure the safe use of probiotics in food products and human applications [17]. Regulations concerning probiotic products vary significantly depending on their intended use. Probiotics marketed as food or dietary supplements are subject to regulations governing food safety and consumer products [18].

4. FERMENTATION ROLE IN THE PRODUCTION OF PROBIOTIC FOODS

Fermentation is one of the oldest methods of food preservation, which has recently regained attention due to its ability to enhance the nutritional and functional properties of food [19]. Foods produced through the microbial transformation of primary and secondary food components, or fermented foods, continue to impact human health [20]. In addition to serving as a means of extending the shelf life of food products and making them safer or more suitable for human consumption, fermentation can also be considered a strategy for mitigating the food industry's exceptionally high environmental impact [21]. Fermentation was likely initially developed as a method for preserving food (food substrates), but it is believed that many fermented foods and their constituents can have beneficial effects on various aspects of human health, particularly digestive health [22]. Although the fermentation process was originally developed for food preservation, it is valued for enhancing flavor, texture, and nutritional benefits. Beyond preservation, fermentation significantly alters food matrices, increasing digestibility and improving nutrient bioavailability, while introducing beneficial microbial communities, diverse enzymes, and various bioactive metabolites [23]. Probiotics are live microorganisms that confer health benefits on the host when consumed in adequate amounts; in the context of fermented foods, these beneficial bacteria can support gut health, modulate immune responses, and play a role in preventing various diseases [24]. Probiotics affect health in various ways: 1. they alter gut bacteria; 2. they boost immune responses; 3. they produce antimicrobial substances; and 4. they compete with harmful organisms for attachment sites on the intestinal lining [25]. Fermented dairy products, such as yogurt and cheese, are considered major sources of probiotics for humans because they create favorable conditions for their growth and survival. Fermented dairy products are considered an important part of the human diet worldwide [26]. Long-term consumption of fermented dairy products induces profound structural remodeling of the gut microbiota, characterized by increased microbial diversity, optimization of ecological balance, and functional specialization [27]. The fundamental principle guiding the inclusion of probiotics in food products embodies a simple yet profound concept: "Do more good than harm. "This principle emphasizes the importance of utilizing beneficial microorganisms to enhance health outcomes and ensures that their inclusion in food products has no adverse effects on consumers [28]. The diversity of microbial ecosystems involved in fermentation underpins a wide range of biochemical processes responsible for the functional and sensory changes observed in fermented foods. In general, genetic engineering and optimized bioreactor systems are considered the driving forces behind the development of more efficient, scalable, and sustainable fermentation processes [29]. The cultivation of probiotics and controlled fermentation under optimal conditions to achieve the desired viability, stability, and functional properties is illustrated in Figure 1.

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Figure **1** Optimized fermentation process for probiotic production.

5. APPLICATIONS OF PROBIOTICS IN THE PRODUCTION OF FUNCTIONAL FOODS

Probiotics are commonly incorporated into products known as functional foods. With the rising consumer demand for functional foods, the food industry has explored a variety of probiotic-enriched products, including dairy items, non-dairy beverages, fermented foods, and dietary supplements [12]. Probiotic foods have evolved from traditional fermented products into scientifically validated functional foods whose health benefits extend far beyond basic nutrition. Probiotic foods containing live microbial cultures currently fall into two categories: dairy products (such as yogurt and cheese) and non-dairy products (such as fruit juices and cereals) [30]. An examination of probiotics in foods and beverages reveals a world of microscopic heroes. Found in fermented foods and emerging strains, these beneficial microorganisms play a vital role in promoting health and well-being [28]. Various methods exist for incorporating probiotics into food products and maintaining their viability. Newer fortification strategies have been adopted to enhance the potential and utility of probiotics; these compounds are used as additives in soy-fortified green tea (combined with Vitamin D) for weight management products, in fruit juices to boost their health-promoting properties, and as a preventive measure against cancer [13]. Lactobacilli are a remarkably diverse group of bacteria now classified into several genera, many of which still retain the name *Lactobacillus*. Despite their name, this group of lactic acid bacteria includes microorganisms that play a vital role in human health, particularly during the early stages of the development of the body's microbiota and immune system [31]. By exhibiting probiotic properties, LAB play a significant role in the functional food industry; they possess the ability to produce various bioactive metabolites such as gamma-aminobutyric acid, exopolysaccharides, conjugated linoleic acid, bacteriocins, reuterin, and reutericyclin that enhance the nutritional value of food products [32]. Compared to conven-

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tional dietary supplements, the probiotic enrichment of food matrices offers improved microbial stability, bioavailability, and microbe-nutrient interactions [33]. When added to foods, probiotic bacteria can make them healthier and offer beneficial health effects, such as boosting the immune system and preventing cancer, diabetes, and cardiovascular diseases [34]. Foods such as cereal-based products, which have high daily consumption, are good vehicles for probiotic supplements [35]. Probiotics are microorganisms that can function as protective cultures in food products. They help preserve food and extend its shelf life, while also enhancing nutritional value by maintaining optimal gut health [36]. Fermented foods are often mistakenly equated with probiotics. Although they may serve as carriers for probiotics or other biotic substances including prebiotics, synbiotics, and postbiotics specific criteria must be met for a fermented food to be considered biotic. These criteria include documented health benefits, adequate product characterization (down to the strain level in the case of probiotics), and testing [37].

6. PROCESSING, STABILIZATION, AND MAINTENANCE OF PROBIOTIC VIABILITY IN FOOD PRODUCTS

Probiotics have significant health benefits that have led to their increased use in various food products. Their viability is an important factor that affects the nutritional and therapeutic efficacy of the food [38]. For probiotics to exert their beneficial effects on living cells, they must reach their site of action. Therefore, careful selection of the appropriate probiotic formulation is required. The type and strain of probiotics affect their survival in transit through the gastrointestinal tract [39]. Careful selection of probiotic strains and their delivery systems is essential to ensure that sufficient numbers reach their site of action and exert health benefits [40]. With increasing consumer consumption, probiotic formulations containing single and multiple strains of probiotics are widely available in various pharmaceutical and food forms, including capsules, suspensions, sachets, granules, tablets, and aerosol products [10]. The health-improving effect of probiotics requires that the probiotics reach the colon in a live form [41]. The viability of probiotics is very challenging, as they are exposed to numerous hazards during industrial processing, storage, and transportation from therapeutic devices to laboratory sites [42]. Factors that make probiotics useful for consumption significantly reduce their bioavailability. These factors are related to heat, pH, water activity, and oxygen [43]. Encapsulation of probiotics has been shown to be an effective way to address these problems, as it significantly improves their efficacy, stability, and bioavailability [44]. Encapsulating probiotic bacteria protects them from harsh conditions during fermentation. Their wall materials can include alginate, chitosan, gelatin, or plant mucilage, whey proteins, and gums, which are characterized by being cheap and durable, without antimicrobial activity, and suitable for food [45]. To increase the activity and stability of this microorganism, researchers encapsulate probiotics, which allows for targeted release at the site of action and overcomes the challenges associated with the processing and application of probiotics [46].

7. HEALTH-PROMOTING AND FUNCTIONAL EFFECTS OF PROBIOTIC FOOD PRODUCTS

Consumption of foods containing probiotics can have beneficial effects on the digestive system, metabolic processes, antioxidant, anti-inflammatory, anticancer, and psychobiological properties

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[47]. Probiotics exert direct and indirect health effects by altering the composition of the gut microbiome to produce bioactive metabolites [48]. They exert their benefits by altering the microbiota, enhancing intestinal barrier integrity, eliminating pathogens, producing antimicrobial metabolites, and regulating innate and adaptive immune responses [49]. They are beneficial microbes that naturally exist in the human stomach and maintain human health. Probiotics are of great importance, both in the development of industrial products and for human health [50]. Probiotics have benefits in the biological detoxification of food and significantly reduce food contamination [51]. The use of foods containing probiotics can have beneficial effects on the digestive system, metabolic processes, antioxidant, anti-inflammatory, anticancer, and psychobiological properties [47]. Probiotics offer a promising new strategy for the management of neurodegeneration, especially Alzheimer's and Parkinson's [52]. Some probiotic strains have been linked to increased microbial diversity and reduced tumor promoting factors in patients with colon cancer [53]. The use of probiotics has increased widely in recent years in the medical, pharmaceutical, and food industries, and the consumption of probiotic products continues to expand due to the health promoting effects of these microorganisms [47]. Probiotics improve the function of the intestinal barrier by accumulating beneficial bacteria. They regulate immune responses and play a role in improving some systemic diseases. Because they are not destroyed by stomach acid and bile salts, they can maintain their beneficial effects in the digestive tract [54]. A food product classified as a probiotic must contain 10^6 colony forming units (CFU) of probiotic microorganisms per gram. For human consumption, a daily intake of 10^7 to 10^9 CFU is required [55]. Based on the definition of probiotics "live microorganisms which, when administered in adequate amounts, confer a health benefit on the host" established during a joint FAO/WHO expert consultation, it was concluded that the characteristics and potential benefits of probiotics depend on the dosage and cannot be generalized from one microbial strain to another, even if both belong to the same species [56]. Probiotics are generally safe for healthy individuals; however, certain population groups with underlying medical conditions such as systemic infections, harmful metabolic activities, excessive immune system stimulation, or gene transfer are highly sensitive to the consumption of probiotics [55]. According to data from 2020, approximately 733 interventional studies involving the use of probiotics have been registered on ClinicalTrials.gov, aimed at improving conditions such as gastrointestinal dysfunction, mental health disorders, cancer, obesity, viral and bacterial infections, sarcopenia, aging, allergies, metabolic syndrome, periodontal disease, and chronic diseases [57].

8. INDUSTRIAL CHALLENGES IN PRODUCTION AND COMMERCIALIZATION OF PROBIOTIC PRODUCTS

The susceptibility of probiotics under processing conditions and during gastrointestinal transit can cause their instability and also prevent the delivery of probiotics in adequate amounts to observe beneficial effects. In this context, there is a need to develop useful strategies to protect these microorganisms during production, storage and delivery to gastrointestinal tract [4]. The health benefits of prebiotics may be lost during the digestion and storage process and may not be fully realized [58]. Various embedding technologies have been developed to improve the viability of probiotics during processing, storage, transportation, and passage through the digestive tract

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[59]. Probiotics are exposed to adverse effects of processing and storage during transit through the gastrointestinal tract, and their viability is reduced in this process. Encapsulation processes are being developed to improve their survival. This leads to the production of dairy and non-dairy foods without changing their physical and sensory properties, which improves human health [60]. Probiotics can only perform their beneficial clinical role when the number of viable cells reaches more than 10^6 CFU g^{-1} [61]. When probiotic bacteria are exposed to stomach acid and bile salts, their viability is significantly reduced, and only a fraction of the cells survive to reach the large intestine to exert their beneficial effects [39]. In vitro and in vivo evaluation models are essential to evaluate the viability, release behavior, and performance of encapsulated probiotics [62]. In recent years, new studies have shown that using pH-sensitive materials to encapsulate probiotics can improve their passage through the device [63]. Traditional encapsulation methods such as spray drying, freeze drying, extrusion, and emulsification create and enhance probiotics under harsh conditions, but each has limitations that can hinder their use [64]. Spray drying is widely used and convenient due to its high scalability and reasonable cost, but high drying temperatures may negatively affect the viability of probiotics [4]. Freeze drying maintains viability better, but is not suitable for large-scale production due to higher cost and longer processing time [3]. Extrusion and emulsification improve the protection of active ingredients and provide controlled release, but in general their scaleup and industrialization process is associated with greater complexity [62]. Hydrogels are considered one of the main carriers for oral delivery of probiotics due to their biocompatibility, gentle processing, and ability to precisely target the intestine [65]. The main stages of probiotic isolation, evaluation, production, formulation, quality control, consumption, and their health effects are illustrated in Figure 2.

9. THE BENEFITS OF POSTBIOTICS CONSUMPTION VS PROBIOTICS

LAB have received widespread attention as beneficial microorganisms and probiotics due to their numerous health-promoting properties; however, the use of live LAB is not entirely free of safety concerns, and their safety should be evaluated at the strain level [66, 67]. One of the major concerns associated with the use of probiotic LAB is resistance to antimicrobial agents, particularly antibiotics. Some LAB strains may be resistant to clinically important antibiotics, and this resistance may be acquired in some cases. The presence of acquired resistance genes is of particular concern because, theoretically, these resistance determinants may be horizontally transferred to other microorganisms, including pathogenic bacteria [68, 69]. Another important challenge of live probiotics is that their efficacy depends on cell viability.

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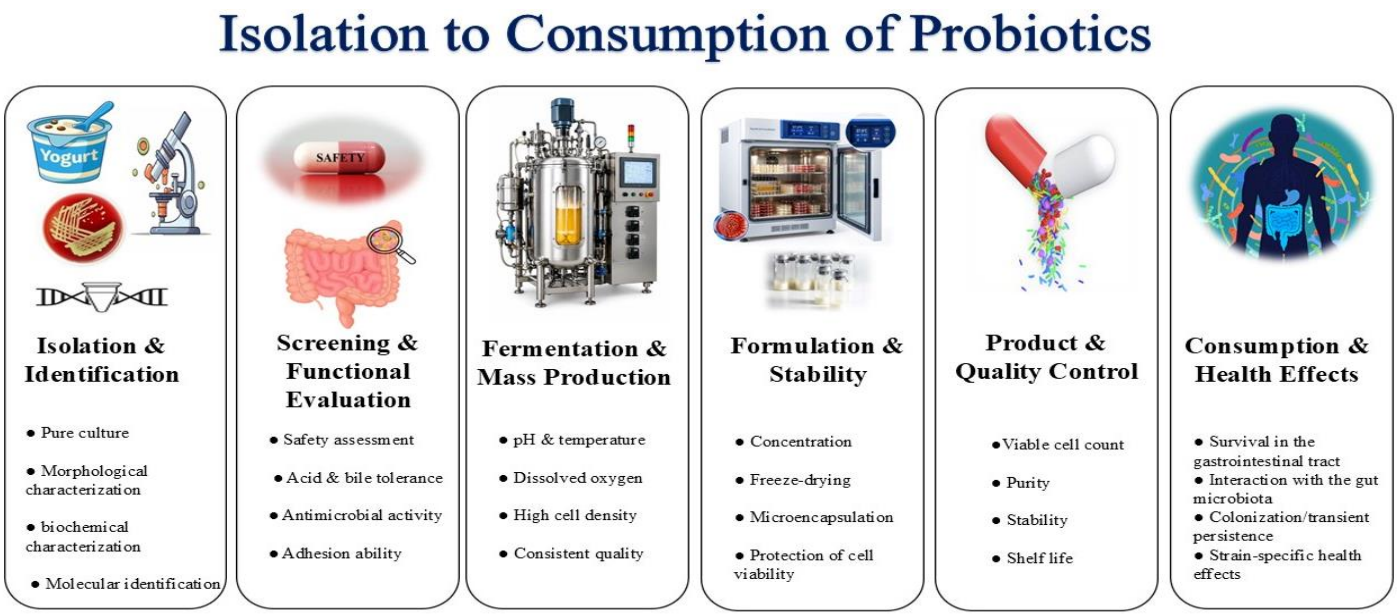


Figure 2 Stages in the development of probiotics, from isolation to consumption and their health effects.

To maintain the effectiveness of probiotic products, a sufficient number of viable cells must remain throughout processing, storage, and consumption [3]. LAB may be sensitive to various environmental factors, such as temperature, oxygen, moisture, and pH. In addition, following oral administration, probiotic cells must tolerate adverse gastrointestinal conditions, including gastric acidity and bile salts, and, when required, reach their site of action. The ability of these strains to colonize or persist may also vary among different strains and individuals [70, 71]. Postbiotics have gained increasing attention in recent years as an alternative or complementary approach to live probiotics. Postbiotics comprise preparations of inactivated microorganisms and/or their components that can confer beneficial effects on the host [72, 73]. Since postbiotics do not require microorganisms to remain viable and replicate, certain bioactive compounds produced by LAB can be delivered to the host without administering live cells [72, 74]. Depending on the producing strain and production method, these preparations may contain inactivated cells, cell wall components, proteins, peptides, exopolysaccharides, organic acids, bacteriocins, and other bioactive metabolites [72, 74, 75]. Postbiotics may exhibit greater stability than live probiotics because they do not require living cells, but their bioactive compounds are still affected by processing and storage conditions [76]. Their composition and biological activity depend on several factors, including the producer strain, inactivation method, and production conditions, which complicates standardization between studies [72]. Although inactive cells lack the ability to proliferate and colonize, this dose not necessarily mean they are inherently safer than probiotics. Their safety depends on factors such as cytotoxicity, type of impurities, and dosage; also, standard protocols for assessing their safety are currently limited [77]. Currently, clinical evidence related to postbiotics is limited and heterogeneous; therefore, more carefully designed human studies are needed to prove efficacy and determine the dose-response relationship [72]. One potential advantage of postbiotics is the reduction of some safety concerns associated with the consumption of live microorganisms [78]. Since the cells in postbiotic

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preparations are inactivated, their ability to replicate and colonize is substantially reduced. This feature may reduce concerns related to bacterial translocation and opportunistic infections, making postbiotics a suitable option for populations in which the use of live microorganisms requires greater caution [76,78]. Unlike live probiotics, postbiotics do not depend on maintaining a specific number of viable cells during processing and storage to exert their biological activity. So, these preparations may exhibit greater stability and, under certain conditions, provide longer shelf life and greater formulation flexibility [76,78,79]. The challenges associated with live probiotics and the potential advantages of postbiotics are compared in Table 2.

Table 2. Challenges of live probiotics and advantages of postbiotics.

Challenges of Probiotics	Benefits of Postbiotics	References
Antibiotic resistance	No requirement for viable cells	[69, 72, 74]
Dependence on cell viability	Greater stability	[3, 77–79]
Sensitivity to processing and gastrointestinal conditions	Longer shelf life	[70, 71, 77–79]
Potential bacterial translocation and opportunistic infections	Reduced concerns related to infections	[77, 78]
Limited formulation flexibility due to viability requirements	Greater formulation flexibility	[77–79]

10. FUTURE PERSPECTIVES

Jessing stresses, and compatibility with diverse food matrices. The broader application of omics technologies, including genomics, could facilitate the identification of strain-specific characteristics and stress-resistance mechanisms, thereby enabling more precise selection of strains suitable for industrial applications [80]. The development of precise and controllable fermentation processes, optimization of culti-vation conditions, and improvement of biomass production yield will be important priorities for future research. Moreover, considering the physiological quality of cells alongside the amount of biomass produced may help enhance probiotic survival during subsequent processing and storage stages [2]. On the other hand, the development of protective technologies and advanced delivery systems, particularly microencapsulation, novel coating materials, and non-thermal methods, can play an important role in preserving the viability and functionality of probiotics during processing, storage, and gastrointestinal transit. Combining these technologies with appropriate food matrix design can facilitate the development of a wider range of probiotic products, particularly non-dairy products [4]. The development of industrial-scale probiotic production will require an integrated approach in which the selection of suitable strains, optimization of production conditions, maintenance of cell viability and functionality, and enhancement of product stability are addressed simultaneously. Advances in biotechnological and process technologies can contribute to the identification of strains with more desirable characteristics, improvement of fermentation processes, and enhancement of probiotic stability under adverse conditions. In addition, the development of novel processing and stabilization methods, together with appropriate food product design, can improve the preservation of probiotic quality and functionality from production to consumption. Ultimately, focusing on the development of scalable, cost-effective, safe, and controllable processes, along with comprehensive evaluation of product stability and quality, can facilitate the translation of laboratory research findings into industrial production and support the development of stable and reliable probiotic products. The food matrix plays a vital role in the successful delivery, survival, and functionality of

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probiotics within functional food products. When well-designed, it ensures the survival of probiotics during processing, storage, and transit through the gastrointestinal tract, while also influencing the sensory characteristics, consumer acceptance, and efficacy of the final product [12]. To ensure consistent health promoting or disease preventing effects, probiotic manufacturing must incorporate appropriate process controls, quality attributes, and operating procedures particularly those parameters that may affect the strains' mechanisms of action [81]. Although a wide variety of probiotic strains are available on the market, the search for new probiotics continues [12,81].

11. CONCLUSON

Probiotics possess considerable potential as functional food products due to their wide range of health-promoting effects, including supporting intestinal barrier integrity, modulating immune responses, maintaining gut microbiota balance, and contributing to antioxidant, anti-inflammatory, metabolic, and potentially anticancer effects. However, the effective application of probiotics largely depends on their ability to maintain sufficient viability and functionality throughout food processing, storage, gastrointestinal transit, and delivery to the target site. Processing and environmental stresses, together with exposure to gastric acid and bile salts, remain major challenges that can reduce probiotic survival and consequently limit their beneficial effects. The development of protective technologies, such as encapsulation, pH-responsive systems, and hydrogel-based carriers, plays an important role in enhancing probiotic stability and facilitating their targeted delivery to the intestine. The selection of appropriate strains, optimization of production processes, and application of advanced protective technologies can contribute significantly to improving the stability and efficacy of probiotic food products. The development of scalable technologies and rigorous evaluation of their efficacy and safety may further facilitate the broader application and commercialization of these products.

12. DECLARATION

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12.2. Data availability

No data was used for the research described in the article.

12.3. Ethics statement

Not applicable.

12.4. Author Contributions Statement

SH: Writing Original Draft, Methodology, Review & Editing, **HF:** Writing Original Draft, **HM:** Writing Original Draft, **MM:** Conceptualization, Methodology, Validation, Investigation, Resources, Writing Original Draft, Writing Review & Editing, Visualization, Supervision, Project administration, **DZ:** Conceptualization, Methodology, Validation, Investigation, Resources, Writing Original Draft, Writing Review & Editing, Visualization, Supervision, Project administration.

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