Journal of Human Environment and Health Promotion

Print ISSN: 2476-5481 Online ISSN: 2476-549X

The Role of Probiotics in Human Health: Significance, Mechanisms of Action, and Factors Influencing Viability

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ARTICLE INFO

Article type:
Review article

Article history:
Received: 13 September 2025
Revised: 23 October 2025
Accepted: 6 November 2025
Available online: 18 November 2025

https://doi.org/10.61882/jhehp.715

Keywords:
Probiotics
Functional foods
Prebiotics
Encapsulation
Synbiotics

ABSTRACT

Background: This review highlights major probiotic strains, challenges in maintaining their viability, and current technological strategies to enhance their stability during processing and storage.
Methods: A comprehensive review was conducted using publications from leading nutrition journals and databases like PubMed and Medline. From an initial 114 records, 49 relevant studies were selected.
Results: Most probiotic formulations include lactic acid bacteria, mainly from the *Lactobacillus* and *Bifidobacterium* genera. Their viability is affected by environmental factors such as high temperatures, stomach acidity, oxygen exposure, and poor storage conditions. Processing techniques like heat treatment, freeze-drying, spray-drying, and the use of specific ingredients also impact survival. To address these challenges, methods such as strain selection, microbial encapsulation, and application of synbiotics have been applied. Evidence supports the role of probiotic-enriched foods in promoting gut microbial balance, alleviating diarrhea, constipation, and symptoms of Irritable Bowel Syndrome and Inflammatory Bowel Disease. Additional benefits include reduced risk of colorectal cancer, improved lactose tolerance, immune modulation, inhibition of *Helicobacter pylori*, enhanced calcium absorption, and reduced serum cholesterol.
Conclusion: Optimizing factors that preserve probiotic viability during food processing and storage is key to ensuring their functionality and health benefits upon consumption.

1. Introduction

Probiotics are recognized as functional food components due to their capacity to deliver health advantages that extend beyond basic nutrition (Palanivelu et al., 2022). Functional foods refer to those products or ingredients that offer additional physiological benefits beyond their standard nutritional roles (Zabetakis et al., 2023). According to the International Scientific Association for Probiotics and Prebiotics (ISAPP), probiotics are 'live microorganisms that, when administered in adequate amounts, confer a health benefit on the host' (Hill et al., 2014). Since these beneficial microbes are typically consumed via food, maintaining their viability until the point of consumption is essential (Küçükgöz & Trząskowska, 2022). For a product to qualify as a probiotic-functional food, it must contain a minimum concentration of 10^7 CFU/mL at the time it is consumed (Palanivelu et al., 2022). The effective dosage needed to influence the gut microbiota is generally within the range of 10^6 to 10^8 CFU/mL or CFU/g (Zabetakis et al., 202).

Among probiotic strains, *Lactobacillus* and *Bifidobacterium* are most commonly utilized, with other genera like *Enterococcus*, *Streptococcus*, and *Leuconostoc* also playing significant roles in product development (Palanivelu et al.,

How to cite: Maina, J. N., Makokha, A., & Aduol, K. (2025). The Role of Probiotics in Human Health: Significance, Mechanisms of Action, and Factors Influencing Viability. *J Hum Environ Health Promot*, *12*(1), 3-12.

2022; Pradhan et al., 2020). Dairy-based probiotic carriers include items such as fermented milk beverages, cheeses, sour cream, ice cream, milk-based desserts, baby formulas, and even chocolate products (Liu et al., 2024). In addition to dairy, probiotics have been incorporated into a variety of other food systems, including meats, cereals, fruits and vegetables, fruit juices, and baked goods (Küçükgöz & Trząskowska, 2022).

Probiotics are involved in numerous physiological processes, such as breaking down harmful substances (xenobiotics), transforming mycotoxins in food, and synthesizing essential vitamins like K, B2 (riboflavin), and folate. They also aid in fermenting undigested fibers in the colon (Średnicka et al., 2021). These microbes inhibit the adhesion of pathogens to the mucosal lining and support immune modulation, thereby strengthening the gut barrier (Nguyen et al., 2023). Consuming probiotics has been associated with improved gastrointestinal health, including reduced incidences of diarrhea, constipation, and symptoms of irritable bowel syndrome (IBS) and inflammatory bowel disease (IBD) (Zhang et al., 2022). Additionally, they have shown promise in reducing allergic reactions, alleviating lactose intolerance, preventing colorectal cancer, regulating immune responses, inhibiting *Helicobacter pylori*, enhancing calcium absorption, and lowering blood cholesterol levels (Chandrasekaran et al., 2024; Gou et al., 2022).

Probiotic strains such as *Lactobacillus*, *Bacillus*, *Bifidobacterium*, certain yeasts, and *Escherichia coli* Nissle 1917 must be capable of withstanding the acidic conditions and bile salts encountered in the human gastrointestinal tract (López-Palestino et al., 2025). Additionally, they should exhibit strong intestinal colonization ability and demonstrate beneficial health effects in clinical studies (Fekete et al., 2024). Nonetheless, these microorganisms are highly susceptible to degradation caused by digestive enzymes, production processes, and storage parameters (Średnicka et al., 2021). Their viability is particularly threatened by the gastrointestinal environment, notably by gastric acids, bile, and enzymes, as illustrated in Figure 1. Strains like *Lactobacillus*, *Bifidobacterium*, and *Pediococcus* have been reported to be sensitive to simulated gastrointestinal conditions (pH 2, for 1 hour at 37°C) (Markowiak-Kopeć & Śliżewska, 2020), highlighting the necessity for maintaining their viability during gastrointestinal transit.

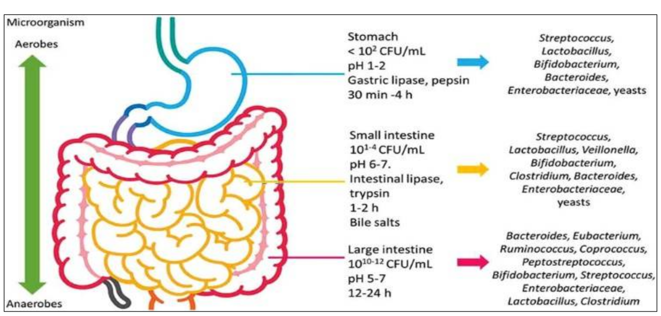

Figure 1. Schematic illustration of the physicochemical environment across various regions of the human gastrointestinal tract (GIT) (Średnicka et al., 2021)

Within the gastrointestinal tract (GIT), probiotics can support digestive processes by facilitating the breakdown of indigestible carbohydrates, leading to the generation of short-chain fatty acids (SCFAs) (Liu et al., 2024). These SCFAs help lower colonic pH levels and suppress the proliferation of pathogenic bacteria. However, to provide health benefits, probiotics must remain metabolically active and reach the gastrointestinal tract in adequate amounts, typically not less than 10^6 colony-forming units (CFU) per gram (López-Palestino et al., 2025). Consequently, choosing an effective microencapsulation method is essential to ensuring their viability throughout gastrointestinal transit.

In food manufacturing, probiotics are often incorporated in freeze-dried form. However, during processing, packaging, and storage, these cells are subjected to numerous stressors, including pH shifts, post-acidification, oxygen exposure, temperature variations, hydrogen peroxide production, and enzymatic limitations (Palanivelu et al., 2022). Maintaining viability under such conditions requires careful selection of the food matrix and optimal design of production and preservation processes.

When selecting probiotic strains for commercial use, it is crucial to assess not only their safety and technological compatibility but also their functional performance (Pradhan et al., 2020). Key functional traits include the strain′s ability to survive and remain stable in food, persist within the gastrointestinal system, and adapt to specific fermentation and storage conditions. The co-use of prebiotics-defined as a substrate that is selectively utilized by host microorganisms, conferring a health benefit, according to ISAPP (Hill et al., 2014)-can further enhance probiotic efficacy and shelf life. Furthermore, combining probiotics with prebiotics such as fructo-oligosaccharides (FOSs) and mannose oligosaccharides (MOSs) can significantly boost their viability, since prebiotics support microbial survival, growth, and metabolic activity (Sbehat et al., 2022).

To ensure prolonged microbial viability, various strategies have been explored. These include selective strain development, microencapsulation technologies, combining probiotics with prebiotics (creating synbiotics), and employing advanced packaging solutions (Zabetakis et al., 2023). According to ISAPP, synbiotics are ′a mixture comprising live microorganisms and substrate (s) selectively utilized by host microorganisms that confers a health benefit on the host′ (Swanson et al., 2020). Selecting appropriate wall materials and encapsulation techniques is essential to protect probiotics from degradation and inactivation under harsh conditions (Zhong et al., 2025). Various encapsulation methods have been developed, including emulsion cross-linking, spray drying, complex coacervation, layer-by-layer (LBL) self-assembly, hydrogel systems, and electrospinning (Agriopoulou et al., 2023). Previous reviews have provided extensive coverage of the incorporation of probiotics into functional food systems. Accordingly, this review synthesizes recent research on the positive impacts of probiotics, emphasizing their role in shaping gut microbiota, regulating immune function, and contributing to disease prevention. It provides an in-depth examination of the underlying mechanisms by which probiotics act, including strengthening the intestinal barrier, producing antimicrobial agents, and influencing host immune responses. In addition, the article addresses critical factors affecting probiotic viability and effectiveness, such as formulation techniques, storage stability, and the conditions within the gastrointestinal tract. This comprehensive overview serves as a valuable resource for researchers and healthcare practitioners aiming to apply probiotics more effectively in health interventions.

2. Materials and Methods

An extensive literature review was performed using the MEDLINE (PubMed) electronic database. The search strategy incorporated Boolean operators "AND" and "OR" in conjunction with the following keywords: *probiotics*, *functional food products*, *prebiotics*, *enhanced cell viability*, *encapsulation*, and *synbiotics*, along with their various combinations. The inclusion criteria were limited to original research articles written in English and published between 2018 and 2024.

The initial database query yielded a total of 114 articles matching the selected keywords and descriptors. Duplicate records were subsequently removed based on predefined eligibility standards. After applying the exclusion criteria, 49 articles met the final selection requirements for inclusion in this review. Each article′s abstract was carefully examined to ensure relevance and the presence of pertinent data. The chosen studies were then categorized according to specific parameters, including title, authorship, publication year, source journal, study sample, methodology, and key findings.

3. Results and Discussion

3.1 Selection of Probiotic Strains

The human gut is home to over 500 bacterial species, many of which are classified as probiotics. Predominantly, these belong to the *Lactobacillus*, *Bifidobacterium*, and *Enterococcus* genera (Zhang et al., 2022). Commonly found *Lactobacillus* strains in European yogurt products include *L. acidophilus*, *L. johnsonii*, *L. crispatus*, *L. paracasei*, *L. rhamnosus*, *L. helveticus*, and *L. casei* (Ruiz-Ramírez et al., 2023). Other widely utilized probiotic microorganisms include *Lactobacillus plantarum*, *L. reuteri*, *L. lactis*, *L. delbrueckii subsp. bulgaricus*, *Enterococcus faecium*, *Streptococcus thermophilus*, and various species from *Bifidobacterium* such as *B. lactis*, *B. infantis*, *B. longum*, and *B. brevis*, along with strains from *Leuconostoc*, *Pediococcus*, and *Lactococcus* (El-Hosseny et al., 2025). The yeast *Saccharomyces boulardii* is also acknowledged as a probiotic due to its ability to prevent travel-related diarrhea and manage *Clostridium difficile* infections (McFarland & Li, 2024). Probiotic formulations may consist of either single-strain or multi-strain cultures (Chandrasekaran et al., 2024).

The selection of probiotic candidates typically depends on functional efficacy, technological suitability, safety, and physiological traits as illustrated in Table 1 (Pradhan et al., 2020). Effective strains must tolerate harsh gastric conditions, including low pH, bile, and phenolic compounds. Additionally, they should demonstrate bile salt deconjugation capability (Palanivelu et al., 2022). Adhesion to intestinal mucosa, suppression of harmful microbes like *Helicobacter pylori*, *Salmonella* spp., *Listeria monocytogenes*, and *Clostridium difficile*, along with anticancer and anti-mutagenic activity, are also critical factors (Nguyen et al., 2023).

FAO/WHO standards require these organisms to have a safety profile appropriate for their intended use, often designated as Generally Recognized as Safe (GRAS) (Średnicka et al., 2021). The GRAS (Generally Recognized As Safe) designation, assigned by the U.S. Food and Drug Administration (FDA), confirms that a substance-such as a food additive, ingredient, or microorganism-is safe for its intended use (Lefevre et al., 2017). This classification can be based on a long-standing history of consumption before 1958 or supported by scientific evidence (McFarland & Li, 2024). In the case of probiotics, GRAS status indicates that specific strains are deemed safe for use in foods or dietary supplements under defined conditions (Takeda et al., 2023). Common GRAS-approved probiotic strains include *Lactobacillus acidophilus* NCFM, *Lactobacillus rhamnosus* GG (ATCC 53103), *Lactobacillus casei* Shirota, *Lactobacillus plantarum* 299v, *Lactobacillus reuteri* DSM 17938, *Bifidobacterium longum* BB536, *Bifidobacterium bifidum, Bacillus subtilis* DE111, *Streptococcus thermophilus, Saccharomyces boulardii* CNCM I-745, *Bifidobacterium breve* M-16V, and *Bifidobacterium animalis* subsp. *lactis* BB-12, among others (Lefevre et al., 2017; Takeda et al., 2023). These strains have been acknowledged as safe for consumption based on scientific assessments, particularly when used in designated products or within recommended dosage limits. They are widely incorporated into food items and dietary supplements.

Table 1. Summary of selection criteria of probiotic microorganisms

Selection criteria	Source of strains	Key findings	References
Acid/bile tolerance, salt resistance	Egyptian dairy products (*L. delbrueckii, S. thermophiles, P. acidilactici*)	>50% survival at pH 4 and 0.4% bile; potential for local probiotics	(El-Hosseny et al., 2025)
Antibiotic susceptibility, safety	Cocoa fermentation (*Lactobacillus* spp.)	Some intrinsic antibiotic resistance; no virulence factors	(López-Palestino et al., 2025)
Adhesion, antioxidant capacity, and bile tolerance	Human gut and fermented foods (*Lactobacillus* spp.)	Functional differences among isolates by origin	(Zhang et al., 2022)
GRAS/QPS, virulence gene screening	Various *Enterococcus* spp.	*E. faecium* NCIMB 10415 GRAS; other strains under evaluation	(McFarland & Li, 2024)
Acid/bile/NaCl tolerance, antimicrobial	Oral and dietary *Lactobacillus* spp.	Potential for millet-based probiotic applications	(Ruiz-Ramírez et al., 2023)

3.2 Mechanisms of Probiotic Action

Probiotics promote health through multiple biological activities. These include strengthening gut epithelial barriers, outcompeting pathogens, modulating immune responses, and producing neurotransmitters (Figure 2) (Nguyen et al., 2023). Probiotic organisms outcompete harmful microbes for attachment sites and essential nutrients, thereby limiting pathogen colonization (Kaur & Deol, 2020). They also secrete antimicrobial substances such as organic acids, hydrogen peroxide, bacteriocins, and short-chain fatty acids (SCFAs), which reduce pathogen populations in the intestinal tract (Table 2) (Markowiak-Kopeć & Śliżewska, 2020). Additionally, these beneficial microbes improve mucosal integrity by increasing mucin secretion, enhancing the expression of tight junction proteins like occludin and claudin-1, and fine-tuning the immune signaling pathways (Table 2) (Ghosh et al., 2021).

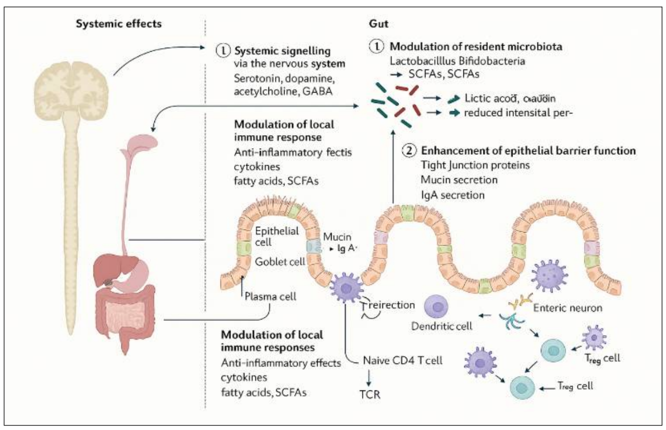

Figure 2. Local (gut-specific) and systemic (whole-body) beneficial effects of probiotics (Nguyen et al., 2023)

Probiotics are also known to influence both innate and adaptive immunity by acting on dendritic cells, macrophages, and lymphocyte populations (Baddouri & Hannig, 2024). They promote the release of anti-inflammatory cytokines and interact with epithelial cells to recruit immune cells such as mononuclear cells and macrophages (Średnicka et al., 2021). Furthermore, through the gut-brain axis, specific probiotic strains can synthesize neurotransmitters like serotonin, dopamine, and GABA, which influence gastrointestinal motility, emotional well-being, and stress regulation (Fekete et al., 2024; Ghosh et al., 2021).

3.3 Beneficial Effects of Probiotics

3.3.1 Modulation of Gut Microbiota

Probiotic supplementation contributes positively to the host's gut microbial composition and overall health status (Chandrasekaran et al., 2024). In studies involving fermented oatmeal with *Lactobacillus*, increased populations of *Lactobacilli* were observed in the gut mucosa, while levels of gram-negative anaerobes such as *Enterobacteriaceae* and sulfite-reducing *Clostridia* were reduced (Gou et al., 2022). These probiotics limit harmful bacteria by occupying binding sites and secreting antimicrobial compounds like SCFAs, hydrogen peroxide, and bacteriocins (Table 3) (Küçükgöz & Trząskowska, 2022).

Consumption of *Lactobacillus GG* resulted in elevated fecal counts of the probiotic and decreased concentrations of fecal enzymes like α-galactosidase and nitroreductase, as well as urinary toxins like p-cresol, commonly produced by non-lactic acid bacteria such as *Bacteroides fragilis* (Chandrasekaran et al., 2024). Moreover, infants fed *B. bifidum*-supplemented formula exhibited higher levels of this probiotic in their feces and had lower pH values compared to those fed standard formula (Gou et al., 2022). These findings reinforce the conclusion that probiotics support a healthier gut flora and improve host health outcomes.

Table 2. Summary of recent studies highlighting the mechanisms of action of probiotics

Study Focus	Probiotic Strains	Mechanisms of Action	Key Findings	References
Gut Health & Barrier Function	*Lactobacillus plantarum, L. reuteri, Bifidobacterium longum* BB536	Enhances tight junction proteins; modulates TLRs, NF-kβ, and MAPK pathways	Improved intestinal epithelial integrity; decreased proinflammatory cytokines	(Ghosh et al., 2021)
Immune Modulation	*L. casei, B. breve, L. rhamnosus* GG	Induces Tregs, promotes IL-10 secretion, boosts IgA and mucin production	Reduced inflammation and enhanced immune tolerance	(Mazziotta et al., 2023)
Antimicrobial Activity	*Lactobacillus acidophilus, Bacillus subtilis, Lactobacillus plantarum*	Produces bacteriocins and SCFAs; inhibits adhesion and virulence genes of pathogens	Effective against *E. coli, S. aureus, Clostridium difficile,* and Salmonella	(Nguyen et al., 2023)
Gut-Brain Axis & Cognitive Function	*L. rhamnosus* GG, *B. longum, Bifidobacterium infantis*	Modulates neurotransmitters (GABA, serotonin), reduces HPA axis activity, and alters gut microbiota	Improved mood, cognition, and reduced anxiety in animal and clinical studies	(Fekete et al., 2024)
Metabolic Health & Short Chain Fatty Acid (SCFA) Production	*L. plantarum, B. breve, Faecalibacterium prausnitzii*	Increases butyrate and propionate; regulates lipid metabolism and insulin sensitivity	Beneficial in models of obesity, hyperlipidemia, and T2DM	(Liu et al., 2024; Markowiak-Kopeć & Śliżewska, 2020)
Oral Health & Periodontitis	*L. reuteri, L. rhamnosus*	Competes with oral pathogens; reduces inflammation; stabilizes oral microbiota.	Decreased plaque index and gingival bleeding in clinical trials	(Baddouri & Hannig, 2024)
Detoxification of Environmental toxins	Mixed strains (e.g., *L. rhamnosus, B. bifidum*)	Binds BPA, phthalates, and microplastics; reduces oxidative stress and inflammatory cytokines	Reduced toxic burden in experimental models; potential protective effect against IBD	(Średnicka et al., 2021)

3.3.2 Management of Diarrhea

Numerous investigations have established the beneficial role of probiotics in managing diarrhea caused by bacterial infections, rotavirus in children, and gastroenteritis in both infants and adults (Săsăran et al., 2023). Probiotic strains such as *Bifidobacterium longum*, *Enterococcus* SF, *Lactobacillus acidophilus*, *L. bulgaricus*, *L. rhamnosus* GG, *Streptococcus faecium*, and *Saccharomyces boulardii* have demonstrated effectiveness in reducing bacterial-induced diarrhea (García-Santos et al., 2023). For pediatric patients experiencing rotavirus-induced diarrhea or gastroenteritis, the administration of *Bifidobacterium bifidum*, *L. rhamnosus* GG, *Enterococcus* SF, *L. casei*, *L. reuteri*, *S. boulardii*, and *S. thermophilus* has proven beneficial (Table 4) (Săsăran et al., 2023).

While limited research has been conducted on traveler's diarrhea, some findings suggest that *L. rhamnosus* GG, *S. boulardii*, and a mix of *Lactobacillus*, *Bifidobacterium*, and *Streptococcus* strains can offer significant relief. Conversely, *L. acidophilus*, *L. bulgaricus*, *L. fermentum* KLD, or their combinations showed no considerable therapeutic impact (Huang et al., 2021). Furthermore, combining the antibiotic vancomycin with *S. boulardii* was associated with a 16.7% reduction in recurrence rates of *Clostridium difficile* infections (Collinson et al., 2020).

Table 3. Summary of recent studies highlighting the beneficial effects of probiotics across various health conditions

Health benefit	Study type	Key findings	Reference
Modulation of gut microbiota	Clinical trial/Review	Probiotics restored gut microbiota balance, increased beneficial bacteria, and improved gut barrier function.	(Chandrasekaran et al., 2024; Gou et al., 2022)
Management of diarrhea	Meta-analysis	Probiotics significantly reduced the duration and severity of acute infectious diarrhea in children and adults.	(Collinson et al., 2020)
	Systematic review and meta-analysis	Probiotics significantly reduced the duration of acute diarrhea and the length of hospital stays in children.	(Huang et al., 2021)
Irritable Bowel Syndrome (IBS)	Randomized Controlled Trial	Specific multispecies probiotics improved abdominal pain, bloating, and bowel movement frequency in IBS patients.	(Matsuura et al., 2024)
Inflammatory Bowel Disease (IBD)	Systemic Review	Probiotics reduced inflammation and maintained remission in ulcerative colitis patients.	(Lopes et al., 2021)
	Systematic review and meta-analysis	Probiotics reduced clinical symptoms, decreased serum inflammatory markers like C-reactive protein, and increased beneficial gut flora in patients with IBD.	(Jakubczyk et al., 2020)
Cancer risk reduction	Experimental and clinical	Probiotic strains showed potential to inhibit colon cancer cell growth and modulate carcinogen metabolism.	(Davoodvandi et al., 2021)
	Clinical trial	Consuming at least two servings of yogurt per week was associated with a 20% reduction in the risk of Bifidobacterium-positive proximal colon cancer, a particularly deadly type of colorectal cancer.	(Ugai et al., 2025)
Immunomodulation	Clinical trial	Probiotic supplementation enhanced immune response and reduced the incidence of respiratory infections.	(Du et al., 2022; Lehtoranta et al., 2020)
Cholesterol-lowering effects	Meta-analysis	Regular probiotic intake modestly reduced total and LDL cholesterol in hypercholesterolemic individuals.	(Shimizu et al., 2015)
	Meta-analysis	Probiotic supplementation effectively reduced total cholesterol, triglycerides, and low-density lipoprotein cholesterol (LDL-C) levels. No significant effect on high-density lipoprotein cholesterol (HDL-C) levels.	(Jiang et al., 2020; Sivamaruthi et al., 2021)

3.3.3 Alleviation of Symptoms of IBS and IBD

Irritable Bowel Syndrome (IBS) typically presents with symptoms like abdominal discomfort, bloating, altered bowel habits, and occasionally systemic issues such as fatigue or nausea. Some probiotic strains, including *L. plantarum* DSM 9843, *L. plantarum* 299V, and *Enterococcus faecium* PR88, have shown promise in reducing IBS symptoms (Matsuura et al., 2024). Inflammatory Bowel Disease (IBD), involving chronic intestinal inflammation primarily in the ileum and colon, manifests through symptoms such as abdominal pain, persistent diarrhea, and in some cases, anemia and fever (Koirala & Anal, 2021). In teenagers, supplementation with *Lactobacillus GG* significantly improved IBD outcomes (Lopes et al., 2021). Additionally, in adults, a six-month regimen of *S. boulardii* (1 g/day) reduced relapse rates from 37.5% to 6.25% (Koirala & Anal, 2021). Ex vivo studies demonstrated that co-culturing inflamed ileum tissue from IBD patients with *L. casei* or *L. bulgaricus* inhibited TNF-α secretion. Consuming 100 mL/day of milk fermented with *Bifidobacterium* significantly lessened ulcerative colitis episodes and reduced *Bacteroidaceae* levels (Table 3) (Jakubczyk et al., 2020).

3.3.4 Cancer Risk Reduction

Probiotics have been implicated in cancer prevention through various mechanisms (Kaur & Deol, 2020). These include immune modulation, carcinogen degradation, modulation of gut microbiota, synthesis of anti-mutagenic substances, alteration of microbial metabolism and colonic pH, and changes in host physiological functions (Mazziotta et al., 2023). Specific strains like *Lactobacillus*, *Bifidobacterium*, and *S. thermophilus* have shown efficacy in minimizing gene mutations (Table 3) (Palanivelu et al., 2022).

Meta-analyses confirm that probiotics help prevent diarrhea caused by cancer treatments like chemotherapy and radiotherapy (Palanivelu et al., 2022). Volunteers consuming diets rich in fried meat showed reduced urinary and fecal mutagenicity after taking *L. acidophilus*. Similarly, administering *L. acidophilus* and *B. bifidum* to individuals with colon adenomas curtailed mucosal cell proliferation in the colonic crypts (Davoodvandi et al., 2021). In vitro studies revealed that strains like *L. casei*, *L. plantarum*, *L. rhamnosus*, *L. acidophilus*, *L. delbrueckii*, and *B. bifidum* offer protection against the genotoxic agent 4-NQO (Davoodvandi et al., 2021). Probiotic-induced reduction in gut transit time through stool bulking further minimizes colon carcinogen exposure (Ugai et al., 2025).

3.3.5 Immunomodulation

Probiotics influence both innate and adaptive immunity by interacting with immune cells, including dendritic cells (DCs), macrophages, and T and B lymphocytes (Figure 2) (Kaur & Deol, 2020; Mazziotta et al., 2023). These interactions primarily occur at the gut epithelial interface. By adhering to mucosal surfaces, probiotics block antigen translocation and outcompete pathogens (Ghosh et al., 2021). They also enhance phagocytic function and stimulate nonspecific defenses, increase IgA production, and reinforce the intestinal barrier (García-Santos et al., 2023). *L. brevis* subsp. *coagulans* and yogurt containing live cultures elevate interferon-α levels (Du et al., 2022). Pattern recognition receptors (PRRs) on intestinal cells are activated by probiotics, prompting cytokine release and Treg cell activation, essential for mucosal homeostasis (Table 3) (Ghosh et al., 2021). Gut-associated lymphoid tissue (GALT) mechanisms involve antigen transport by M cells to DCs, which direct naive T cells toward Th1, Th2, Th17, or Treg responses (Du et al., 2022). Each T-helper type plays distinct roles, with Th1 promoting IFN-γ production, Th2 supporting humoral immunity via IL-4/5, Th17 producing IL-17, and Tregs secreting IL-10 or TGF-β (Mazziotta et al., 2023). Probiotics further stimulate B cells to differentiate into IgA-secreting plasma cells, with IgA then migrating into mucus layers to neutralize pathogens (Lehtoranta et al., 2020). *L. johnsonii* LJ-1 and *L. salivarius* UCC 118 promote phagocytosis and IgA release. *L. bulgaricus* and *L. casei* regulate Th1/Th2 cytokine balance by modulating TNF-α (García-Santos et al., 2023). Additionally, probiotics such as *L. plantarum*, *L. lactis*, and *L. rhamnosus* GG can attenuate Th2-mediated allergic responses (Du et al., 2022). They also offer protection during viral respiratory infections by releasing SCFAs, antimicrobial substances, and competing for adhesion and nutrients (Koirala & Anal, 2021). Probiotic metabolites like peptides, vitamins, and amino acids play vital roles in immune modulation (Mazziotta et al., 2023).

3.3.6 Cholesterol-Lowering Effects

Elevated serum cholesterol is a major contributor to cardiovascular diseases. Research involving animal models and human subjects has shown that LAB may lower cholesterol levels (Jiang et al., 2020). One key mechanism involves the inhibition of HMG-CoA reductase by hydroxymethyl-glutarate, reducing cholesterol synthesis (Sivamaruthi et al., 2021). Additional mechanisms include cholesterol assimilation by probiotic membranes, conversion to coprostanol, bile salt deconjugation, and production of SCFAs (Shimizu et al., 2015).

L. plantarum PH04 (4×10^8 CFU/mL) significantly reduced lipid absorption and boosted lipid metabolism in test subjects (Jiang et al., 2020). Probiotic yogurt containing *L. acidophilus* and *Bifidobacterium* (10^6 CFU/mL) lowered serum cholesterol (Sivamaruthi et al., 2021). Conversely, a study using capsules of *L. acidophilus* DDS-1 and *B. longum* UABL-14 found no such effect, suggesting the delivery matrix significantly impacts probiotic viability in the gut (Jiang et al., 2020).

3.4 Survival Assessment and Viability of Probiotics

Probiotic viability pertains to the ability of bacterial cells to grow and reproduce under specific environmental conditions (Palanivelu et al., 2022). Successful delivery of probiotic benefits relies heavily on the ability of selected strains to survive passage through the gastrointestinal environment and endure the processing stages during food production (Fiore et al., 2020). Numerous investigations have emphasized the importance of viable microbial cells in achieving desired probiotic effects. More recently, innovative technologies are being explored to maintain or enhance probiotic viability throughout product storage, as illustrated in Table 4. Probiotic populations often experience a significant reduction-sometimes by factors of 10 to 100 or greater-during manufacturing and storage (Palanivelu et al., 2022). Factors such as oxygen levels, thermal exposure during heating or fermentation, acidity, moisture levels, osmotic pressure, and type of packaging materials all play crucial roles in maintaining microbial survival (Fadiji et al., 2023; Sbehat et al., 2022). Various strategies, including vacuum packaging, incorporation of antioxidants, or use of oxygen scavengers like ascorbic acid, are employed to mitigate oxygen exposure in packaged probiotics (Palanivelu et al., 2022). Oxygen reduction during fermentation can also be achieved by anaerobic processing conditions, enzymatic oxygen scavenging, or through genetic modification of probiotic strains such as *Bifidobacterium* (Fiore et al., 2020).

Storage conditions significantly affect probiotic stability. Key food-related factors include pH, total acidity, oxygen content, water activity, salt and sugar concentrations, and presence of additives such as hydrogen peroxide, bacteriocins, and artificial preservatives (Yang et al., 2024). Meanwhile, process-related factors such as heating steps, incubation and cooling protocols, packaging approaches, and production scale also influence microbial survival (Palanivelu et al., 2022). Additionally, biological aspects-such as the probiotic strain′s inherent resistance, interactions with starter cultures, existing microflora, enzyme activity, acid production post-fermentation, and presence of spoilage or pathogenic microbes-impact cell viability (Fadiji et al., 2023).

To evaluate bacterial vitality in probiotic foods, two primary methodologies are used. Culture-based techniques, which measure colony-forming units per gram (cfu/g), remain the gold standard for quantifying viable cells (García-Santos et al., 2023). However, alternative culture-independent methods, including DNA-based assays and flow cytometry, are increasingly utilized and refined for accurate assessment of microbial presence without requiring cell cultivation (Palanivelu et al., 2022).

3.5 Strategies for Enhanced Probiotic Viability

3.5.1 Selection of Food Packaging Systems

The survival of probiotics can be significantly influenced by the characteristics of packaging materials and the methods used for packaging (Fiore et al., 2020). Most dairy-based probiotic products are commonly packaged in plastics that allow considerable oxygen transmission. Since some probiotic strains, particularly anaerobic bacteria like *Bifidobacteria*, are highly sensitive to oxygen exposure, packaging with high oxygen permeability can reduce their viability during storage (Fadiji et al., 2023). Upadhyay et al. (2024) demonstrated improved survival of *Lactobacillus acidophilus* in yogurt stored in glass bottles compared to plastic, recommending thicker packaging materials to better preserve both *L. acidophilus* and *Bifidobacteria* in dairy products (Table 4).

Fiore et al. (2020) examined probiotic viability in yogurt packaged in various plastic containers differing in oxygen permeability, along with the addition of glucose oxidase. As anticipated, containers with lower oxygen transmission rates supported higher probiotic survival, despite increased post-acidification and organic acid accumulation. Therefore, packaging with reduced oxygen permeability generally promotes the longevity of probiotic cultures (Fadiji et al., 2023). However, glass containers, although effective in limiting oxygen ingress, tend to raise packaging costs (Upadhyay et al., 2024). Cost-effective alternatives include the incorporation of oxygen scavengers or absorbers, vacuum packaging, or the use of active packaging materials with enhanced oxygen barrier properties.

3.5.2 Addition of Compounds as Probiotic Enhancers

Various substances can be incorporated into probiotic formulations to support bacterial growth or to protect them during processing. These include growth-promoting agents such as sugars, vitamins, minerals, and prebiotics, as well as protective ingredients like skim milk powder, whey proteins, glycerol, and lactose (Gharibzahedi & Smith, 2021). Mudgil et al. (2024) evaluated the potential of casein glycomacropeptide (GMP) hydrolysates as prebiotic additives in yogurt, noting a positive effect on the proliferation of *Streptococcus thermophilus* but a lesser impact on *Lactobacillus bulgaricus*. Parhi et al. (2022) observed that adding fructo-oligosaccharides (FOS) to skim milk boosted the survival of commercial *Bifidobacterium* species by 55.7% after four weeks of refrigerated storage. Similarly, incorporating 1.5% (w/v) oligofructose as a prebiotic into yogurt helped maintain probiotic viability throughout storage (Parhi et al., 2022). Other protective compounds, including whey protein hydrolysate, inulin, and whey protein concentrate, have also been shown to enhance probiotic survival (Table 4) (Gharibzahedi & Smith, 2021).

3.5.3 Development of Synbiotics

Synbiotics refer to products that combine probiotics with prebiotics to improve the survival and colonization of beneficial bacteria in the gastrointestinal tract (Sbehat et al., 2022). While probiotics primarily exert their effects in both the small and large intestines, prebiotics mainly influence the large intestine. Their combination can result in synergistic benefits (Liu et al., 2024). The stimulation of probiotics by prebiotics helps regulate intestinal metabolic activities, supports the integrity of intestinal structures, fosters the growth of advantageous microbial populations, and suppresses harmful pathogens. For example, Săsăran et al. (2023) developed synbiotic milk chocolate using encapsulated *Lactobacillus casei*, demonstrating that inulin-enhanced chocolate can serve as an effective probiotic delivery system. In vivo studies on mice fed synbiotic milk chocolate showed increased fecal lactobacilli counts, reduced coliform bacteria, and lower α-glucuronidase activity (Liu et al., 2024). Furthermore, Sbehat et al. (2022) formulated prebiotic, probiotic, and synbiotic ice creams, with the synbiotic versions containing inulin and various *Lactobacillus* strains (Table 4).

3.5.4 Probiotic Encapsulation

Microencapsulation refers to the technique of enclosing probiotic cells within tiny protective capsules to shield them from unfavorable external conditions (Palanivelu et al., 2022). This approach aims primarily to safeguard probiotics against environmental stresses. Encapsulation improves the stability of probiotic cultures, simplifies their handling and storage, and protects delicate lactic acid bacteria from factors such as oxygen exposure, freezing temperatures, and acidic environments encountered during production, storage, and digestion (Table 4) (Agriopoulou et al., 2023).

The effectiveness of encapsulation depends on several variables, including the encapsulation method used, the type of wall material, the pH of the environment, the initial density of probiotic cells, the bacterial strain, and the characteristics of the food matrix (Yang et al., 2024). Common encapsulating agents include polysaccharides such as starch, alginate, various plant or microbial gums, chitosan, and cellulose acetate phthalate; proteins like gelatin and milk proteins; and certain lipids (Palanivelu et al., 2022). Techniques frequently applied to encapsulate probiotics include spray drying, freeze-drying, vacuum drying, and the formation of gel-based microspheres (Table 4) (Agriopoulou et al., 2023).

Table 4. Summary of recent studies highlighting the technologies for enhanced probiotic viability

Technology	Description	Probiotic strains	Key findings	References
Single-Cell Encapsulation Techniques	Encapsulates individual probiotic cells using advanced materials for targeted delivery and improved survival	Lactobacillus, Bifidobacterium species	Improved protection against environmental stressors; potential for precise delivery in the gastrointestinal tract.	(Zhong et al., 2025)
Immobilization on Bacterial Cellulose	Probiotics are immobilized on bacterial cellulose matrices, improving their viability in functional dairy beverages.	*Lactiplantibacillus pentosus*, *Lactiplantibacillus plantarum*	Enhanced viability during storage; improved sensory properties in fermented products	(Yang et al., 2024)
Drying Techniques (freeze-drying and Spray-drying)	Applies drying methods to dehydrate probiotics, affecting their viability during storage and digestion.	Various probiotic strains	Spray-dried probiotics showed higher viability under in vitro digestion compared to freeze-dried ones.	(Agriopoulou et al., 2023; Yang et al., 2024)
Encapsulation Techniques	Double/Multiple Layer Beads: Utilizing materials like alginate, chitosan, and whey protein isolate to form multi-layered beads. Electrospinning: Creating nanofibers with polymers such as polyvinyl alcohol (PVA) and bacterial cellulose nanofibers (BCNF) High Internal Phase Emulsions (HIPEs): Stabilized with whey protein isolate microgels	*Lactobacillus acidophilus* Various probiotic strains	Enhances probiotic protection against gastrointestinal conditions; microcapsules provided effective protection during spray drying. HIPEs significantly enhance the viability of *Lactobacillus plantarum* during food processing.	(Zhong et al., 2025)
Synbiotic Development	Co-Encapsulation: Simultaneous encapsulation of probiotics with prebiotics like inulin or fructo-oligosaccharides Microencapsulated Synbiotics: Combining probiotics with prebiotics in microcapsules	*Lactiplantibacillus plantarum* NCIMB 11974	Optimal FOS concentration (2%) promoted probiotic growth; microcapsules (~ 230 µm) ensured protection and controlled release. Enhances survival rates during storage and digestion, offering synergistic health benefits.	(Sbehat et al., 2022; Liu et al., 2024)
Probiotic Enhancers	Protein-Polysaccharide Conjugates: Encapsulation using pea and rice protein-inulin conjugates Natural Fibers: Utilizing jackfruit inner skin fiber as an encapsulating material	Various probiotic strains	Significantly improves the viability and stability of *Lactobacillus reuteri* during storage. Enhances probiotic stability against adverse conditions.	(Mudgil et al., 2024; Gharibzahedi & Smith, 2021)
Food Packaging Systems	Active Packaging Solutions: The integration of antimicrobial compounds and oxygen-absorbing elements into packaging materials Eco-Friendly Biodegradable Films: Employing biodegradable films infused with probiotics	Various probiotic strains	Enhances the stability and viability of probiotics throughout storage. Provides an environmentally sustainable method for preserving and delivering live probiotic cultures via packaging.	(Fadiji et al., 2023; Upadhyay et al., 2024)

4. Conclusion

Probiotics, primarily lactic acid-producing bacteria, are recognized as functional foods because they offer health advantages beyond basic nutrition. They contribute to preventing harmful microbial colonization in the gut, alleviate conditions such as diarrhea and irritable bowel syndrome, enhance immune responses, and may help reduce the risk of colon cancer. Selecting specific probiotic strains is important for targeting particular health outcomes, as different strains vary in their effects. For probiotics to confer health benefits, they must remain viable throughout the product's shelf life. Achieving this requires compatibility among the probiotic strain, the food matrix, production methods, and storage conditions. Incorporating prebiotics, growth-enhancing compounds, and plant-derived ingredients into the matrix, along with optimized processing, fermentation, and storage, can improve probiotic survival. Maintaining an environment with low oxygen levels, controlled moisture, minimal osmotic stress, and effective packaging is critical for maximizing probiotic stability. Microencapsulation has emerged as a promising method to overcome challenges related to probiotic stability in food products. Additionally, combining probiotics with prebiotics such as inulin and oligofructose fosters their growth in the gastrointestinal tract. Scaling probiotic incorporation to industrial levels presents microbiological, technological, and economic challenges. Continued research is essential to develop better technologies, select optimal carrier matrices, and choose bacterial strains that can withstand processing and survive passage through the upper digestive system. Although methods like microencapsulation improve viability, they add costs to food production. Many encapsulation technologies remain underutilized and require further study to enable successful application in commercial food products. Future efforts should focus on enhancing microcapsule properties, broadening available techniques, and addressing production challenges to facilitate the development of novel probiotic foods.

Authors' Contributions

Jane Njeri Maina: Conceptualization; writing-original draft preparation; writing-review and editing. **Anselimo Makokha:** Conceptualization; writing-review and editing; visualization; supervision. **Kevin Aduol:** Writing-review and editing; supervision. All authors have read and agreed to the published version of the manuscript.

Funding

This study did not receive any external financial support.

Conflicts of Interest

The authors declare that there are no conflicts of interest related to this work.

Acknowledgments

The authors sincerely thank the University of Eldoret and Jomo Kenyatta University of Agriculture and Technology for their valuable resources and support throughout this research.

Ethical considerations

Since this review did not involve any experiments with human participants or animals conducted by the authors, ethical approval was not necessary.

Using Artificial Intelligence

No artificial intelligence tools or technologies were employed by the authors during the writing or preparation of this review article.

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