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Probiotics in food: Navigating obstacles and embracing cutting-edge solutions

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Abstract
Probiotics, defined by the FAO/WHO as "live microorganisms that, when administered in adequate amounts, confer a health benefit on the host," represent a burgeoning sector of the global functional food market. Their purported benefits, spanning improved gut health, enhanced immunity, and reduced risk of various non-communicable diseases, have fueled intense consumer interest and industrial innovation. However, the successful integration of these delicate microbial cultures into food matrices faces significant technological and biological obstacles. The primary challenge is maintaining the requisite viability—typically ≥106 to ≥107 Colony Forming Units (CFU) per gram or milliliter—throughout food processing, storage, and passage through the harsh environment of the gastrointestinal (GI) tract. Factors such as low pH, high oxygen concentration, processing temperatures, and interactions within the food matrix drastically compromise probiotic survival. Furthermore, ensuring the safety of novel strains, particularly concerning the transfer of antibiotic resistance genes and potential for systemic infection in immunocompromised individuals, remains a critical regulatory and scientific concern. To overcome these hurdles, cutting-edge solutions are emerging. Next-generation probiotics (NGPs), encompassing strict anaerobes like *Akkermansia* and *Faecalibacterium*, offer new therapeutic potential but demand highly specialized delivery systems. The most promising technological innovation is microencapsulation, utilizing materials like alginate, chitosan, and various hydrogels, which provides a protective barrier against environmental stresses and targeted release in the colon. Other strategies include the development of synbiotics (probiotics combined with prebiotics), the selection of robust, stress-tolerant strains, and the exploration of novel food carriers beyond traditional dairy, such as non-dairy beverages and fermented cereals. This paper reviews the multifaceted challenges in incorporating viable and effective probiotics into food products and details the current and future innovative strategies aimed at enhancing their viability, efficacy, and safety, thereby unlocking their full therapeutic potential in the functional food industry.

Keywords: Probiotics, microencapsulation, next-generation probiotics, para probiotics, food stability, symbiotic

Introduction

The concept of functional foods, which transcend basic nutritional provision to offer distinct health benefits, has driven significant advancements in food science and technology over the past few decades. Within this category, products enriched with probiotics have garnered particular attention. Probiotics—live microorganisms that, when consumed in adequate amounts, confer a health benefit on the host—primarily belong to the *Lactobacillus* and *Bifidobacterium* genera, though the field is rapidly expanding to include yeasts and newer, less-studied bacterial species (Hill *et al*., 2014; Sanders *et al*., 2019) [13, 25]. The benefits associated with their consumption are diverse, ranging from the modulation of gut microbiota composition, competitive exclusion of pathogens, production of short-chain fatty acids (SCFAs), and enhancement of the intestinal epithelial barrier function, to systemic effects such as immune modulation, reduced inflammation, and improved mental health via the gut-brain axis (Plaza-Díaz *et al*., 2019; Hamad *et al*., 2022) [22, 12].

Despite the considerable consumer demand and therapeutic promise, the transition of efficacious probiotic strains from the laboratory bench to commercially successful, viable food products is fraught with complexities. The fundamental challenge lies in maintaining a sufficiently high concentration of live cells (≥106 or ≥107CFU/mL/g) throughout the entire product lifecycle, from initial inoculation and processing to storage, shelf-life, and final transit through the consumer's upper gastrointestinal tract (GIT) (Stavropoulou *et al*., 2020) [28]. The technical obstacles encountered during food manufacturing are multifaceted. Probiotic strains are highly susceptible to the processing conditions of various food matrices. For example, the low pH and high titratable acidity characteristic of fermented dairy products like yogurt, while a traditional carrier, can be detrimental to the viability of many sensitive bifidobacteria during extended storage (Gupta *et al*., 2020; Lucatto *et al*., 2020) [11, 18]. Oxygen exposure during mixing or packaging, especially in non-dairy beverages, can generate reactive oxygen species that are lethal to most anaerobic probiotic species (Gómez-Torres *et al*., 2021) [10]. Furthermore, temperature fluctuations during cold chain distribution and the mechanical stress of processing operations such as homogenization and spray drying further reduce cell counts (Gomes *et al*., 2021, Singh K., 2025) [9, 33].

Beyond technological hurdles, biological challenges related to survival within the human host are equally critical. Upon ingestion, probiotics must survive the extreme acidity of the stomach (pH1.5−3.5) and the presence of bile salts and pancreatic enzymes in the small intestine before reaching the colon, where they are intended to exert their primary beneficial effects (Gomes *et al*., 2021) [9]. Efficacy, therefore, is not merely a matter of initial cell count but of guaranteed strain survival and activity in the target site.

Safety and regulatory concerns also present significant obstacles. These include rigorous strain-specific identification, genetic stability, and the need to rule out transferable antibiotic resistance genes and virulence factors (Sanders *et al*., 2019; Plaza-Díaz *et al*., 2019) [25, 22]. For the next generation of non-traditional probiotic species, such as strict anaerobes and those sourced outside of dairy, establishing a history of safe use and achieving regulatory approval demands extensive and specialized toxicological assessment (O'Toole *et al*., 2017) [21].

The industry is now actively seeking and implementing a range of innovative solutions to address these profound challenges. The search for superior carrier matrices has expanded beyond dairy to encompass non-dairy fermented products (e.g., fruit juices, fermented cereals, and vegetable-based substrates), which cater to specific dietary preferences (vegan, lactose-intolerant) and often offer a higher pH to mitigate acid-related death (Mousavi *et al*., 2021; Fathima *et al*., 2023) [20, 6]. Most notably, advanced delivery technologies, such as microencapsulation and nanoencapsulation, are being rapidly developed to create physical protective barriers for the cells (Gomes *et al*., 2021; Gismondo *et al*., 2021) [9, 8].

This paper aims to provide a comprehensive review of the significant challenges currently facing the incorporation of viable probiotics into food products, from manufacturing to biological efficacy. It will then detail the most promising cutting-edge solutions, including advanced microencapsulation techniques, the application of postbiotics and paraprobiotics, and the emergence of Next-Generation Probiotics (NGPs), which together define the future trajectory of functional food innovation.

Technological Obstacles in Food Formulation and Processing

The core challenge in the functional food sector is maintaining the effective dose of probiotics, typically defined as ≥106 to ≥107 Colony Forming Units (CFU) per gram or millilitre, until the point of consumption (Costa *et al*., 2019; Lucatto *et al*., 2020) [5, 18]. Probiotic viability is aggressively undermined by several factors inherent to food production and storage:

- **Manufacturing Stress:** Processes such as high-shear mixing, homogenization, and thermal treatments (e.g., pasteurization or baking) inflict irreparable mechanical and heat damage on sensitive microbial cells (Gomes *et al*., 2021) [9]. Dehydration methods like spray drying, which are essential for producing stable probiotic powders, often result in significant cell death due to thermal stress and water activity reduction (Gupta *et al*., 2020) [11].
- **Food Matrix Effects:** Many popular food matrices are inherently antagonistic to microbial life. The low pH of fruit juices, fermented dairy, and many sauces is highly detrimental, particularly to *Bifidobacterium* species, which are sensitive to acidic environments (Gómez-Torres *et al*., 2021) [10]. Furthermore, the presence of oxygen, a common feature in non-fermented liquids and solid foods, is a powerful stressor for obligate anaerobes, leading to oxidative damage and mortality (Mousavi *et al*., 2021) [20].
- **Storage and Shelf-Life:** The loss of viability during storage often surpasses the losses during processing. Extended shelf-life requirements, especially for non-refrigerated products, make it nearly impossible for traditional probiotic strains to survive. Factors like residual oxygen, the accumulation of toxic metabolites (e.g., hydrogen peroxide or organic acids), and water activity changes continue to deplete the live cell count over time (Saarela, 2019; Sedaghati & Alizadeh, 2021) [24, 26].

Biological and Regulatory Hurdles

Even if a strain survives the food matrix, it must withstand the harsh environment of the human body and satisfy stringent safety criteria.

- **Gastrointestinal Transit Stress:** The sequential exposure to gastric acidity (pH<3) and high concentrations of bile salts in the small intestine constitutes a lethal barrier for unprotected cells. Only highly acid-and bile-tolerant strains can pass this gauntlet and colonize the colon to exert their function (Gomes *et al*., 2021) [9].
- **Safety and Virulence:** Regulatory bodies demand rigorous, strain-specific safety assessments, including whole-genome sequencing to check for transferable antibiotic resistance genes (ARGs) and virulence factors. The potential for the horizontal transfer of ARGs from probiotic strains to pathogenic or commensal bacteria in the gut is a major concern (Sanders *et al*., 2019; Butel, 2020) [25, 4].
- **Strain Specificity and Efficacy:** The health benefit of a probiotic is highly specific to the strain and dose. Extrapolation of benefits from one strain to another, even within the same species, is scientifically invalid. This requires substantial investment in separate, costly clinical trials for each new functional food product to validate the claimed health effect (Hill *et al*., 2014; Merenstein *et al*., 2021) [13, 19].

Cutting-Edge Solutions and Future Directions

To overcome these obstacles, research and industry efforts are focusing on a three-pronged approach: advanced delivery systems, novel strain development, and the utilization of non-viable microbial components.

Advanced Delivery Systems: Microencapsulation and Synbiotics

Microencapsulation is the most promising technological solution, providing a physical barrier that shields probiotic cells from environmental stresses.

- **Materials and Techniques:** Natural, food-grade biopolymers like alginate, chitosan, pectin, and whey protein are commonly used as wall materials. Techniques such as extrusion (producing larger beads), emulsification, and complex coacervation allow for the formation of microcapsules that are stable in food matrices and resistant to gastric juices, only releasing the cells in the higher pH environment of the intestine (Karimi *et al*., 2020; Varghese *et al*., 2022) [15, 30].
- **Synbiotic Formulations:** The concept of synbiotics, combining probiotics with prebiotics (non-digestible compounds that selectively stimulate the growth/activity of the probiotic), is increasingly leveraged. Co-encapsulating probiotic cells with a prebiotic (e.g., inulin or fructooligosaccharides) provides a nutrient source within the microcapsule, significantly improving viability during storage and enhancing survival and targeted release in the colon (Amorim *et al*., 2023; Sedaghati & Alizadeh, 2021) [1, 26].

Next-Generation Probiotics (NGPs) and Non-Dairy Matrices

The search for superior strains has moved beyond traditional *Lactobacillus* and *Bifidobacterium* to include Next-Generation Probiotics (NGPs).

- **Novel Strains:** NGPs are typically strict anaerobes and key members of a healthy gut, such as *Akkermansia muciniphila* (implicated in metabolic health and gut barrier function) and *Faecalibacterium prausnitzii* (a major butyrate producer with anti-inflammatory properties). The challenge of their extreme oxygen sensitivity is being addressed by microencapsulation and specialized anaerobic fermentation techniques (O'Toole *et al*., 2017; Zommiti *et al*., 2021) [21, 32].
- **Spore-Forming Probiotics:** Strains of *Bacillus* species (e.g., *B. coagulans*) naturally form heat-and pH-resistant spores, making them ideal for incorporation into baked goods, retorted beverages, and non-refrigerated foods. Their use bypasses many viability issues but requires thorough investigation into their gut activity (Arellano-Hurtado *et al*., 2022) [3].
- **Non-Dairy Carriers:** Driven by consumer demand, the industry is moving towards non-dairy matrices like fruit and vegetable juices, fermented cereals, and plant-based milks. These matrices can offer higher pH values and unique nutrient profiles that may favour the growth and stability of certain strains (Mousavi *et al*., 2021; Fathima *et al*., 2023) [20, 23].

Paraprobiotics and Postbiotics

An emerging paradigm shifts the focus from live cells to non-viable microbial components, offering a solution to viability, stability, and safety concerns.

- **Paraprobiotics (Inactivated Probiotics):** These are non-viable microbial cells (intact or ruptured) that, when administered in adequate amounts, confer a health benefit. They are easier to store, have a longer shelf-life, and pose no risk of ARG transfer or infection. Their effects are often mediated by cell wall components, conferring anti-inflammatory and immunomodulatory benefits (Tuomola *et al*., 2019; Zommiti *et al*., 2021) [29, 32].
- **Postbiotics (Metabolites):** These are functional bioactive compounds—such as short-chain fatty acids (SCFAs), organic acids, bacteriocins, and exopolysaccharides—released by the live microorganisms or resulting from microbial cell lysis. Postbiotics eliminate all viability challenges and can be incorporated into almost any food matrix (Yang *et al*., 2020) [31]. The incorporation of postbiotics, especially SCFAs, into functional foods represents a future direction to deliver targeted health benefits without the complexity of maintaining live cultures.

Table 1: Summary of Key Probiotic Challenges and Corresponding Solutions

Challenge Category	Specific Obstacle	Cutting-Edge Solution	References
Technological/Food Matrix	Low viability during processing (heat/shear)	Microencapsulation (e.g., alginate, chitosan) using extrusion or emulsion methods	Karimi *et al*., 2020 [35]; Varghese *et al*., 2022 [30]
Technological/Food Matrix	Oxygen toxicity and low pH during storage	Use of Non-Dairy Carriers (e.g., fermented fruit/cereal bases) or Synbiotics	Mousavi *et al*., 2021 [20]; Fathima *et al*., 2023 [23]
Biological	Low survival in the gastrointestinal tract (GIT)	Co-encapsulation with Prebiotics (Synbiotics) for targeted release in the colon	Amorim *et al*., 2023 [1]; Sedaghati & Alizadeh, 2021 [26]
Biological/Strain	Sensitivity of next-generation strains (e.g., *Akkermansia*)	Specialized Anaerobic Fermentation and use of Spore-Forming Probiotics (*Bacillus* spp.)	O'Toole *et al*., 2017 [21]; Arellano-Hurtado *et al*., 2022 [3]
Safety and Stability	Risk of infection and loss of viability/shelf-life	Incorporation of Paraprobiotics (inactivated cells) or Postbiotics (metabolites)	Yang *et al*., 2020 [31]; Zommiti *et al*., 2021 [32]

Conclusion

Navigating the integration of probiotics into the food supply requires overcoming significant technological hurdles, primarily centered on preserving cell viability during processing, storage, and gastrointestinal transit. The industry is currently embracing cutting-edge solutions to meet this challenge. Advanced microencapsulation techniques, the formulation of optimized synbiotics, and the exploration of stable spore-forming or Next-Generation Probiotics (NGPs) are paving the way for a new generation of functional foods. Crucially, the rise of paraprobiotics and postbiotics provides a means to deliver defined health benefits without the complexities of maintaining live cultures, promising greater stability, wider food application, and a more robust regulatory pathway.

Data Availability: No new data were generated or analyzed in this study. All information presented is derived from previously published sources, which are appropriately cited in the manuscript.

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