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Biotechnological Production of Probiotic Functional Foods and Their Antimicrobial Effects against Foodborne Pathogens

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Abstract

The integration of advanced biotechnology into functional food production has revolutionized the development of probiotic-enriched products that deliver targeted health benefits while providing robust biocontrol against foodborne pathogens. This review synthesizes recent progress in metabolic engineering, synthetic biology (including CRISPR/Cas9), precision fermentation, and multi-omics-driven strain selection to create next-generation probiotics (NGPs) with enhanced viability, stress tolerance, and bioactive metabolite production (e.g., bacteriocins, organic acids, SCFAs). Valorization of agro-industrial residues through optimized fermentation further enhances sustainability and nutritional value. Innovative delivery systems microencapsulation (extrusion, emulsion, spray drying), nanotechnology, and 3D food printing ensure high probiotic survival (>10^8 CFU/mL) through processing, storage, and gastrointestinal transit. Probiotic functional foods exert potent antimicrobial effects via multiple mechanisms: production of antimicrobial peptides (e.g., nisin, pediocin), pH reduction, competitive exclusion, and biofilm interference, achieving significant log reductions (2–8 log) against major pathogens including Listeria monocytogenes, Salmonella Typhimurium, Escherichia coli O157:H7, and Staphylococcus aureus across dairy, plant-based, and meat matrices. The review also addresses matrix diversification (dairy, cereal, fruit, vegetable, and fermented meat products), market trends toward personalized and precision nutrition, regulatory frameworks (EFSA QPS, FDA GRAS), and safety considerations. Collectively, these biotechnological advancements position probiotic functional foods as sustainable, multi-functional solutions for improving food safety, gut health, and global nutrition security.

Keywords: Probiotic Functional Foods, Biotechnological Production, Precision Fermentation, Synthetic Biology, Crispr/Cas9, Next-Generation Probiotics, Microencapsulation, 3d Food Printing, Antimicrobial Peptides, Bacteriocins, Foodborne Pathogens, Synbiotics, Personalized Nutrition, Agro-Residue Valorization

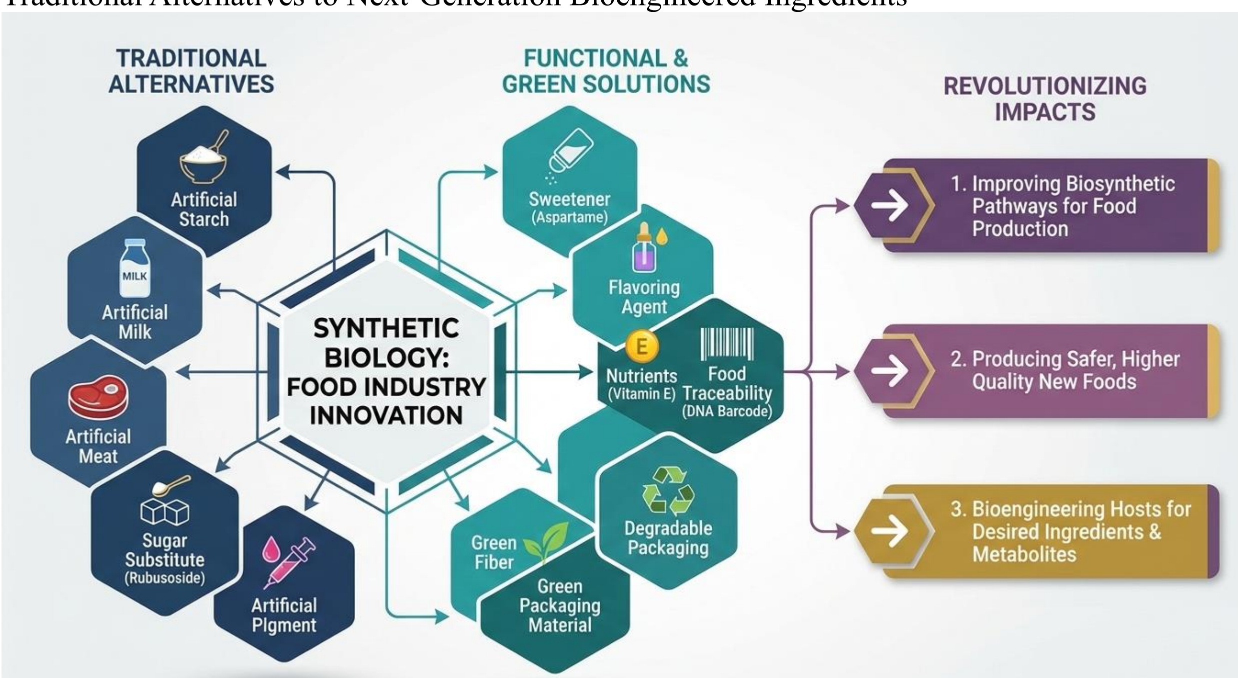
1. Introduction

The contemporary global food landscape is currently witnessing a paradigm shift from traditional caloric sufficiency toward a sophisticated "food as medicine" model (Koberinski et al., 2022). This transition is underpinned by the rapid advancement of biotechnological production methods for probiotic functional foods, which are designed to deliver targeted physiological benefits while simultaneously acting as natural barriers against foodborne pathogens (HDIN Research, 2025). Probiotics, defined as live microorganisms that confer health benefits when administered in adequate amounts, are no longer confined to the dairy aisle; they are being integrated into complex matrices ranging from plant-based beverages to fermented meat products (IUFoST, 2025). This transformation is driven by a fusion of synthetic biology, metabolic engineering, and precision fermentation, which allows for the creation of next-generation probiotics (NGPs) with enhanced stability, viability, and antimicrobial potency (Alenazy et al., 2024).

2. The Biotechnological Evolution of Probiotic Strains

The production of high-performance functional foods requires more than the mere addition of microbial cultures. It necessitates a deep understanding of the molecular crosstalk between the probiotic, the food matrix, and the host's gut environment (Razzak et al., 2026). Biotechnology provides the essential toolkit for optimizing these interactions, moving away from spontaneous fermentation toward a precision-engineered approach that ensures consistency and efficacy (Bali et al., 2024).

Figure 1. Strategic Applications of Synthetic Biology in Food Industry Innovation: From Traditional Alternatives to Next-Generation Bioengineered Ingredients



2.1 Metabolic Engineering and Synthetic Biology Platforms

Metabolic engineering offers a sustainable and precise alternative to conventional chemical synthesis for producing bioactive compounds in the food sector (Singh, 2024). By leveraging synthetic biology, researchers can modify the genetic architecture of traditional probiotic species, such as Lactobacillus and Bifidobacterium, to overproduce specific health-promoting metabolites like vitamins, short-chain fatty acids (SCFAs), and antimicrobial peptides (AMPs) (Yu et al., 2025). The implementation of these technologies contributes to alleviating resource limitations by substituting traditional agricultural extraction with microbial biomanufacturing approaches (Thakur et al., 2023).

For instance, the use of CRISPR/Cas9 technology has emerged as a versatile tool for carrying out permanent and efficient changes in the genomes of probiotic organisms (Vaid et al., 2022). In species such as Lacticaseibacillus rhamnosus, genetic engineering has been employed to enhance stress resistance mechanisms, ensuring the bacteria can survive the industrial rigors of food processing and the subsequent harsh environment of the gastrointestinal tract (Bustos, 2024). Furthermore, engineered probiotics are being designed to act as therapeutic delivery vehicles, capable of releasing anti-inflammatory agents or enzymes directly to the gut mucosa to treat conditions like obesity, type 2 diabetes, and non-alcoholic fatty liver disease (Tang et al., 2021).

2.2 Precision Fermentation and Valorization of Agro-Residues

Fermentation technology serves as the backbone for enhancing food characteristics and nutritional value. Modern biotechnological approaches now focus on the valorization of agro-industrial wastes, converting low-value byproducts into nutrient-dense functional ingredients (Hamouda & Jiang, 2025). This process involves pre-treatment methods physical, chemical, and biological that break down complex substrates, allowing probiotics to thrive and produce novel biologically active molecules (Biotechnological potential, 2024).

Beyond basic fermentation, precision fermentation utilizes microbial hosts as cellular factories to produce specific proteins or lipids that mimic those found in dairy or meat (Sustainable et al., 2026). This is particularly relevant in the production of plant-based functional foods, where microbial proteases are used to improve protein digestibility and reduce allergenicity in cereals and legumes (Avelar et al., 2024). The strategic use of enzymes in these systems enhances the taste, texture, and aroma of the final product while maximizing the bioavailability of micronutrients (Jin et al., 2022).

2.3 Multi-Omics and Bioinformatics in Strain Selection

The identification and characterization of potential probiotic strains have been revolutionized by high-throughput multi-omics platforms (Kwoji et al., 2023). Whole-genome sequencing (WGS) is now required by regulatory bodies like the European Food Safety Authority (EFSA) to monitor genes of concern, such as virulence factors and transmissible antibiotic resistance (AMR) genes (EFSA Scientific Committee, 2025).

Table 1: Applications of Omics Technologies in Probiotic Research

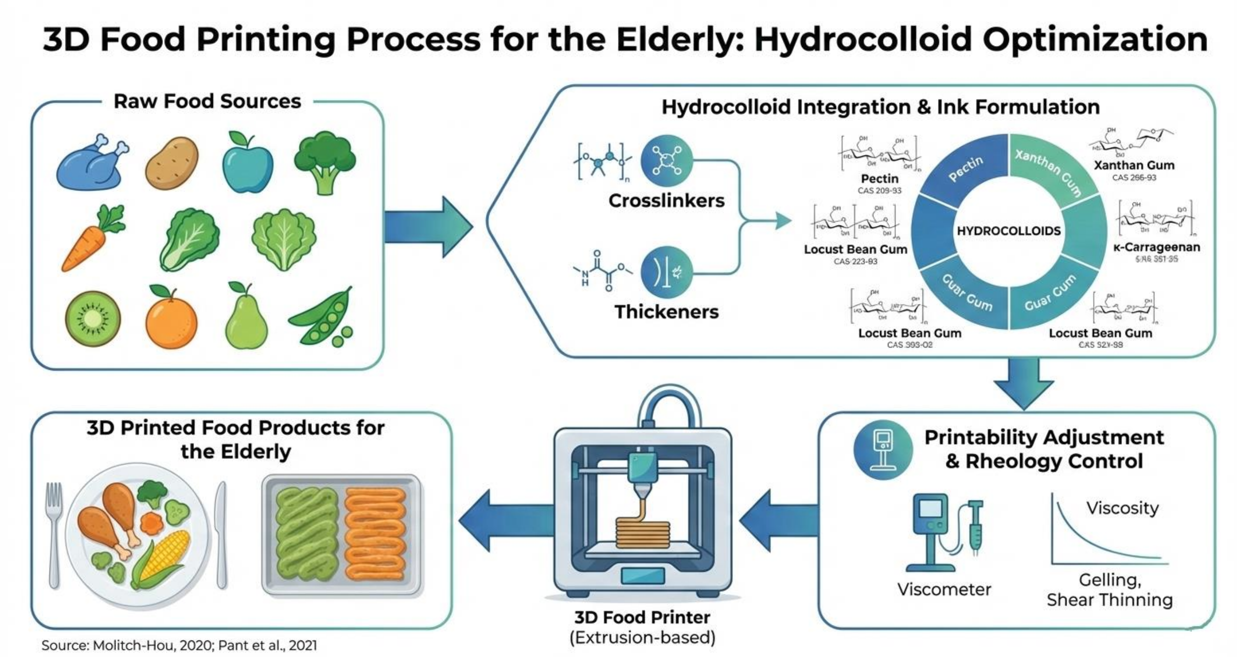
Omics Technology	Application in Probiotic Research	Key Insights Provided
Genomics (WGS)	Taxonomic classification and safety profiling	Detection of AMR genes and virulence factors (EFSA Scientific Committee, 2025)
Transcriptomics	Analysis of host-microbe crosstalk	Modulation of tight junction proteins and cytokines (Folia Microbiologica, 2025)
Proteomics	Evaluation of protein-level expression	Identification of adhesion factors and enzymes (Folia Microbiologica, 2025)
Metabolomics	Profiling of microbial metabolites	Detection of SCFAs, organic acids, and biosurfactants (Folia Microbiologica, 2025)

Bioinformatic tools and machine learning platforms, such as iProbiotics and ProbioMinServer, facilitate the rapid identification of probiotic properties from bulk genomic data, enabling the selection of tailor-made strains for specific health applications (Sun et al., 2022).

3. Innovative Delivery and Protection Technologies

One of the primary challenges in functional food production is maintaining the viability of probiotics during storage and gastrointestinal transit (Palanivelu et al., 2022). Biotechnological innovations in encapsulation and 3D printing have addressed these limitations, providing protective structures that ensure the delivery of a sufficient dose (greater than 10^8 CFU/mL) to the target site (Yu, 2025).

Figure 2. Schematic Workflow of the 3D Food Printing Process: Hydrocolloid Optimization and Rheology Control for Personalized Nutrition in the Elderly



3.1 Microencapsulation Techniques and Materials

Microencapsulation isolates probiotic cells within a semipermeable membrane, shielding them from environmental stresses like low pH, oxygen, and digestive enzymes (Wijegunawardhana, 2023).

Table 2: Comparison of Probiotic Microencapsulation Methods

Encapsulation Method	Common Materials	Mechanism of Release	Industrial Relevance
Extrusion	Alginate, Pectin	Diffusion / Dissolution	Small-scale / High viability (Wijegunawardhana, 2023)
Emulsion	Chitosan, Whey Protein	pH-dependent degradation	Scalable / Flexible size (Wijegunawardhana, 2023)
Spray Drying	Maltodextrin, Gum Arabic	Physical rupture	High throughput / Shelf-stable (HDIN Research, 2025)
Coaxial Printing	Starch core / Alginate shell	Triggered release	Personalized / Precision dosing (Yu, 2025)

The extrusion method remains popular due to its simplicity, utilizing sodium alginate as a hydrocolloid that gels upon exposure to calcium ions. For industrial scalability, the emulsion technique is often preferred, as it allows for the adjustment of capsule size and provides high survival rates through the use of secondary coatings like chitosan (Bufalini & Campardelli, 2025).

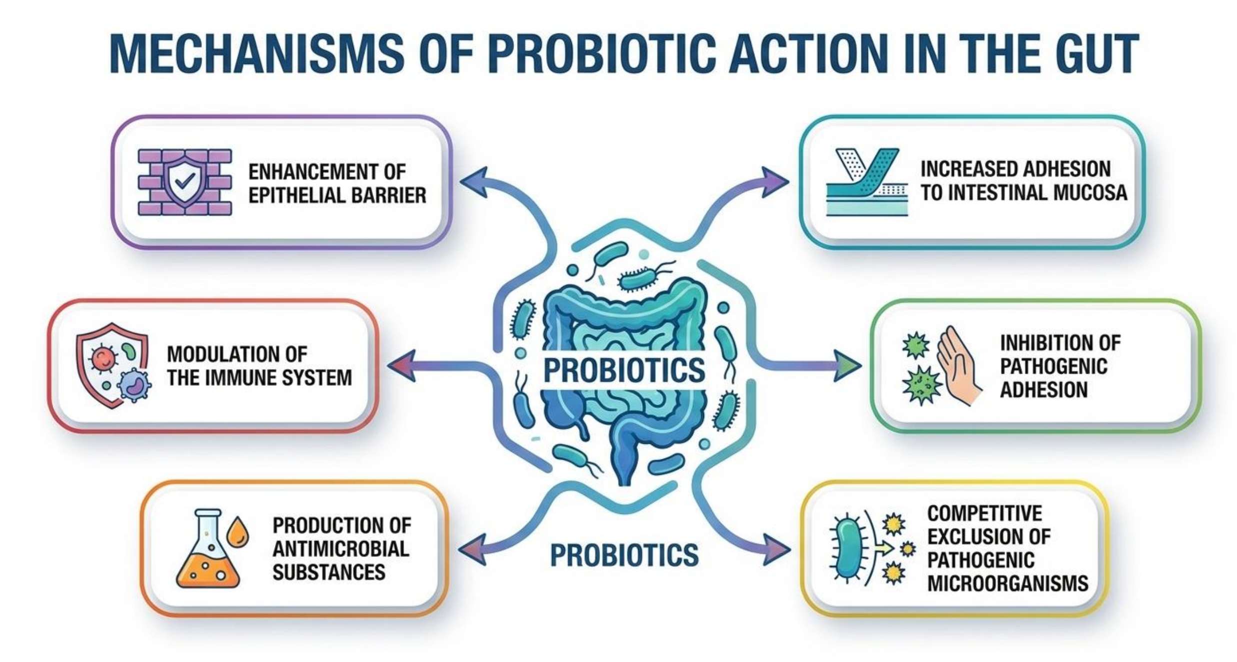
3.2 Nanotechnology and 3D Food Printing

The convergence of nanotechnology and functional foods has led to the development of nanospheres and nanoparticles (dimensions below 1 micrometer) that further improve nutrient absorption and extend product shelf life (Han et al., 2025). Three-dimensional (3D) food printing has emerged as a transformative platform for personalized nutrition (Yu, 2025). Unlike conventional capsules, 3D printing allows for the digital design of edible matrices with programmable geometry. Coaxial and gel-in-gel architectures can buffer local pH and reduce the diffusion of bile salts, retaining over 90-96% of probiotic cells after printing (Navara, 2023).

4. Antimicrobial Efficacy Against Foodborne Pathogens

A defining characteristic of probiotic functional foods is their ability to mitigate the risks posed by foodborne bacterial pathogens through a multi-faceted antimicrobial strategy. This biocontrol function is increasingly valuable as antibiotic resistance (AMR) continues to undermine conventional food safety measures (Vesković Moračanin et al., 2025).

Figure 3. Multifaceted Mechanisms of Probiotic Action in the Gastrointestinal Tract: Enhancing the Epithelial Barrier and Inhibiting Pathogenic



4.1 Mechanisms of Pathogen Suppression

Probiotics employ several distinct yet synergistic mechanisms to inhibit the growth and virulence of pathogens like Listeria monocytogenes, Salmonella Typhimurium, and Escherichia coli O157:H7 (Song et al., 2020).

- **Antimicrobial Peptides (AMPs) and Bacteriocins:** Ribosomally synthesized peptides like nisin and pediocin disrupt bacterial cell membranes by forming pores or inhibiting cell wall biosynthesis (Beyer, 2025).
- **Organic Acid Production:** The secretion of lactic and acetic acids lowers the pH of the food matrix, creating a hostile environment for spoilage organisms (Ibrahim et al., 2021).
- **Competitive Exclusion and Biofilms:** Probiotics colonize food surfaces or the intestinal lining, forming protective biofilms that outcompete pathogens for nutrients and adhesion sites (Deng et al., 2020).

- **Synergistic "Collegial" Strategy:** The integration of live probiotics and their metabolites offers enhanced efficacy by simultaneously attacking pathogens through pore-forming peptides, acidification, and nutrient competition (Vinayamohan et al., 2024).

4.2 Case Studies in Food Matrices

The antimicrobial activity of probiotics has been extensively validated across various food categories, demonstrating significant log reductions in pathogen populations (El Far et al., 2024).

Table 3: Reported Efficacy of Probiotic Strains Against Foodborne Pathogens

Food Matrix	Probiotic Strain	Target Pathogen	Reported Efficacy
Dairy (Kefir)	Potential Probiotic LAB	L. monocytogenes	2.33-2.4 log reduction (Bali et al., 2024)
Dairy (Kefir)	Potential Probiotic LAB	E. coli	2.03-2.4 log reduction (Bali et al., 2024)
Meat (Poultry)	L. plantarum s61	L. monocytogenes	Significant inhibition (Parada Fabián et al., 2025)
Meat (Salami)	L. acidophilus	S. aureus	Over 8-fold reduction (Bali et al., 2024)

In dairy kefir, artificial intelligence-based models have confirmed that probiotic LAB levels remain high while reducing targeted pathogens throughout fermentation and storage (Bali et al., 2024).

5. Matrix Diversity and Technological Adaptations

While dairy remains the dominant carrier for probiotics, the industry is diversifying into non-dairy alternatives to address lactose intolerance and the demand for plant-based diets (Wijegunawardhana, 2023).

5.1 Dairy and Hybrid Systems

Dairy products like yogurt and kefir provide a naturally protective matrix due to their fat and protein content, which buffers probiotics during digestion (Kaur et al., 2022). Modern innovations include the co-encapsulation of plant extracts within dairy matrices to increase bacterial survival and add antioxidant properties (Mehra et al., 2022).

5.2 Plant-Based Functional Foods

Plant-based sources such as cereals, fruits, and vegetables offer unique advantages. They often contain prebiotics like inulin, which provide a "synbiotic" effect, enhancing the survival of probiotic strains (Sionek et al., 2025). For example, fermentation of oat-based drinks increases protein digestibility and B-complex vitamins, while fermented carrot juice has shown a 30-fold increase in iron solubility (Kumar et al., 2022).

5.3 Meat-Based and Ready-to-Eat (RTE) Muscle Foods

The incorporation of probiotics into meat products, particularly fermented sausages, allows for the production of functional foods with high protein content. Probiotic starter cultures assist in rapid acidification, which is critical for safety in salami-type products (Munekata et al., 2022). Furthermore, antimicrobial edible coatings based on chitosan and alginate are being developed for RTE seafood to extend shelf life (Sirini et al., 2022).

6. Global Market Trends and Personalized Nutrition

The probiotics market is entering a high-growth decade, driven by a shift toward preventive healthcare and "precision nutrition" (Food Research Lab, 2025).

6.1 Market Dynamics and Regional Growth

The global probiotics market reached approximately 86 billion USD in 2025 and is projected to expand to 144 billion USD by 2030. Probiotic foods currently hold the majority share (over 53%) of the market (Mordor Intelligence, 2026). Asia-Pacific stands as the global leader in growth potential, while North America and Europe move toward "medicalized marketing" and strain-specific formulations (HDIN Research, 2025).

6.2 Precision Nutrition and NGPs

Future functional foods will be matched to an individual's unique gut microbiota profile using sequencing and machine learning. Next-generation probiotics (NGPs) such as Akkermansia muciniphila are at the center of this trend, offering targeted mechanisms for metabolic regulation (Next-generation probiotics, 2026).

7. Regulatory Frameworks and Safety Standards

As biotechnology advances, the safety assessment of probiotic functional foods has become more rigorous to ensure public health protection (The Business Research Company, 2026).

7.1 EFSA and FDA Guidelines

In the EU, EFSA's 2025 guidance has harmonized the safety assessment of probiotics, regardless of genetic modification status. The QPS status can be extended to certain GMMs if safety criteria are met (EFSA Scientific Committee, 2025). In the US, probiotics must be approved as food additives or be Generally Recognized As Safe (GRAS) (Thakur et al., 2023).

7.2 Safety Concerns

While generally safe, probiotics can lead to adverse effects like bacteremia in compromised individuals. Careful strain selection and genomic screenings for antibiotic resistance genes are essential to prevent the spread of AMR within the host's microbiota (Pandey et al., 2025).

8. Conclusions

Biotechnological advancements have transformed probiotic functional foods from simple fermented dairy products into sophisticated, precision-engineered matrices capable of delivering consistent health benefits and powerful antimicrobial protection against foodborne pathogens. Through metabolic and genetic engineering, multi-omics-guided strain optimization, advanced encapsulation and 3D-printing technologies, and strategic valorization of agro-industrial wastes, these products now achieve superior probiotic viability, targeted bioactive metabolite production, and broad-spectrum pathogen suppression via synergistic mechanisms including bacteriocins, organic acids, competitive exclusion, and biofilm disruption. The diversification into non-dairy, plant-based, and meat-based carriers, combined with the emergence of next-generation probiotics and personalized nutrition approaches, addresses contemporary consumer demands for sustainability, inclusivity, and individualized health solutions. As the global probiotics market continues its rapid expansion, rigorous regulatory oversight (EFSA QPS and FDA GRAS frameworks) and comprehensive safety assessments remain essential to mitigate risks such as antibiotic resistance gene transfer. Future success will depend on scaling precision fermentation and delivery technologies, conducting large-scale clinical trials to validate strain-specific efficacy, and fostering interdisciplinary collaboration between microbiologists, food technologists, and data scientists. Ultimately, biotechnologically enhanced probiotic functional foods represent a cornerstone of the "food as medicine" paradigm offering a safe, natural, and sustainable strategy to combat foodborne illness, strengthen gut health, and promote long-term public wellness in an era of increasing antimicrobial resistance and lifestyle-related diseases.

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