Food Science and Engineering
https://ojs.wiserpub.com/index.php/FSE/

Review

Unculturable Microorganisms in Fermented Foods: New Technological Insights and Emerging Health Applications

Phu-Tho Nguyen[1,2], **Huu-Thanh Nguyen**[1,2*]

[1]Department of Biotechnology, An Giang University, An Giang, Vietnam
[2]Vietnam National University, Ho Chi Minh City, Vietnam
E-mail: nhthanh@agu.edu.vn

Received: 24 June 2025; **Revised:** 4 September 2025; **Accepted:** 20 October 2025

Abstract: Fermented foods harbor Unculturable Microorganisms (UMs) that shape flavor formation, safety, and health-relevant functions. Recent advances—multi-omics, single-cell genomics, culturomics, and Artificial Intelligence (AI)-driven modeling—are uncovering their metabolic roles and ecological importance. Beyond describing their hidden diversity, UMs open new futures for fermented food innovation: redesigning starters to enhance amino nitrogen while reducing precursors of ethyl carbamate and biogenic amines; developing stable UM-derived postbiotics such as extracellular vesicles for immune modulation; applying AI-guided community design to improve flavor consistency and microbial resilience; and creating personalized synbiotic strategies aligned with consumer microbiomes. Gut-on-chip validation and precision fermentation enable scalable translation, while a metabolite-centered safety framework provides a feasible regulatory path for non-culturable taxa. Framing UMs around deployable functions—rather than cultivability—positions them as key levers for next-generation probiotics, postbiotics, and precision fermented foods.

Keywords**:** fermented food, multi-omics, probiotics, postbiotics, unculturable microorganisms

1. Introduction

Fermented foods represent some of the oldest and most diverse microbial ecosystems associated with human nutrition. Traditionally crafted through artisanal practices, these foods harbor complex consortia of bacteria, yeasts, and filamentous fungi that drive the biochemical transformations responsible for enhanced flavor, texture, and shelf stability. While early research mainly focused on culturable organisms capable of growth on standard laboratory media, advances in culture-independent methods have uncovered a broader spectrum of microbial diversity, including a significant proportion of Unculturable Microorganisms (UMs) [1, 2].

UMs are defined as microorganisms that cannot be isolated or propagated using conventional culture techniques, often due to their dependence on specific ecological interactions, stringent nutrient requirements, or unique physicochemical niches [3]. Although long overlooked, these elusive members are now increasingly recognized for their functional significance. Metagenomic and metatranscriptomic analyses have uncovered their involvement in essential fermentation processes, including enzymatic transformations, bioactive metabolite production, and flavor compound biosynthesis [4, 5]. Pinpointing the role of UMs in fermented foods requires linking their functional signatures to measurable product attributes. Multi-omics analyses can trace UM-derived transcripts or enzymes to increases in amino

DOI: https://doi.org/10.37256/fse.6220257580

nitrogen in soybean pastes [6] or the suppression of ethyl carbamate precursors in soy sauce [7]. Metabolomic profiling has correlated UM-associated volatile compounds with flavor development in vinegar and fish sauces [8], while Extracellular Vesicles (EVs) and Short-Chain Fatty Acids (SCFAs) provide markers of immunomodulatory or safety-related effects [1, 5]. Such correlations demonstrate that UMs, although uncultivable, leave identifiable biochemical footprints that directly shape food quality, safety, and health-promoting properties. Establishing these associations provides a practical framework for evaluating UMs not only as hidden microbial community members but also as functional drivers of fermented food characteristics.

The study of UMs has gained renewed importance in the context of emerging trends in microbiome-based health strategies. As consumer interest shifts from generalized probiotic supplementation to personalized, microbiome-driven interventions, UMs are positioned as promising resources for the development of next-generation probiotics, postbiotics, and targeted synbiotic formulations. Their contributions to immune modulation, gut barrier enhancement, and microbial ecosystem stabilization suggest that they may offer health benefits surpassing those of conventional culturable strains [9, 10]. Moreover, technological breakthroughs—such as multi-omics integration, single-cell genomics, high-throughput culturomics, and Artificial Intelligence (AI)-driven modeling—now enable detailed exploration of UMs at genetic, functional, and ecological levels [11-13]. These approaches provide unprecedented opportunities to harness the untapped potential of UMs in functional food innovation, precision nutrition, and microbiome-targeted therapeutics.

This review consolidates current knowledge on UMs in fermented foods, with a focus on modern technological approaches, their emerging roles in human health, and their applications in functional food design. By doing so, it highlights UMs as critical, yet underexplored, players in the evolving landscape of microbiome science and personalized health.

2. Technological breakthroughs in studying UMs

The study of UMs in fermented foods has been revolutionized by advances in multi-omics, single-cell analysis, and computational modeling (Figure 1). These technological breakthroughs provide a comprehensive understanding of microbial diversity, functional roles, and interactions within complex fermentation ecosystems, overcoming the limitations of traditional culture-based methods.

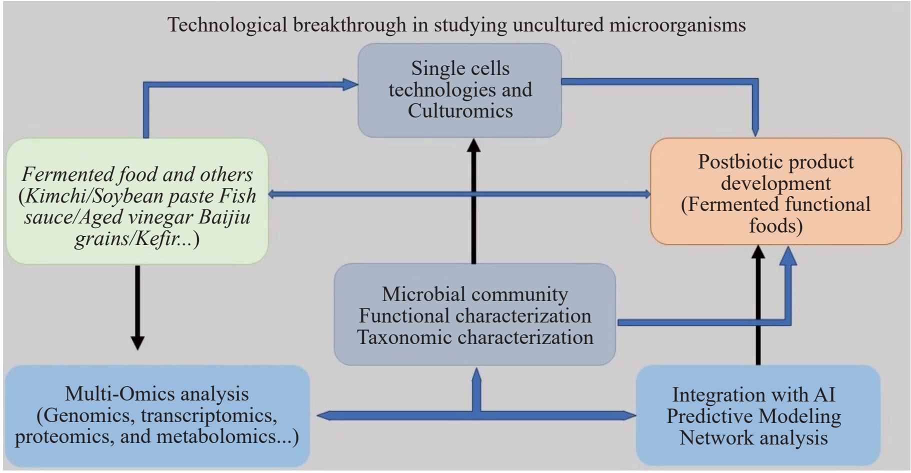

Figure 1. Technological breakthroughs applied to study UMs in fermented foods. Multi-omics platforms, single-cell technologies, culturomics, and AI-driven predictive modeling are increasingly integrated to reveal UM diversity, activity, and functional roles during fermentation

2.1 *Multi-omics platforms*

Metagenomics is foundational for characterizing the microbial diversity of complex ecosystems. By sequencing total community DNA, metagenomics enables the identification of both culturable and unculturable taxa based on conserved genetic markers and whole-genome assemblies. This approach allows for taxonomic classification, estimation of community structure, and prediction of functional gene content without the need for isolation [14, 15]. In fermented foods such as Yucha (a typical traditional fermented food of China), soy sauce, and fish sauce, metagenomics has revealed the presence of novel, uncultured species contributing to substrate degradation, amino acid metabolism, and stress resistance [6, 16, 17]. Metatranscriptomics extends this analysis by profiling actively transcribed genes, offering a dynamic snapshot of functional activity within microbial communities. This method can determine which UMs are metabolically active during different fermentation stages and link gene expression to biochemical transformations such as acidification, volatile production, or bioactive synthesis [18, 19]. In systems like fermented soybean pastes and kimchi, metatranscriptomic data have uncovered transcriptionally active, uncultured bacteria involved in carbohydrate fermentation, vitamin biosynthesis, and lactic acid production [20, 21].

Metaproteomics focuses on identifying and quantifying microbial proteins expressed during fermentation. This method can validate gene expression data from metatranscriptomics and associate enzymatic activities with specific taxa, including UMs. Functional proteins linked to proteolysis, stress response, and metabolite transport have been attributed to uncultured microbes in traditional fermented foods [22]. It also enables detection of extracellular enzymes and signaling molecules involved in microbial interaction and flavor development [23]. Metabolomics complements the above approaches by profiling the small molecules produced by microbial communities, including potential postbiotic compounds. These include SCFAs, bacteriocins, peptidoglycans, and other secondary metabolites with health-promoting or preservative properties [24]. In fermented foods, metabolomic signatures can be mapped to specific metabolic pathways of UMs inferred from multi-omics datasets. Notably, targeted metabolite profiling during kimchi fermentation revealed that different capsaicinoid concentrations influenced the dynamics of Lactic Acid Bacteria (LAB) , free sugars, amino acids, and microbial communities, suggesting a metabolite-microbiome interaction shaped by red pepper cultivar [25]. Together, these omics layers form an integrated system biology approach to understanding UMs—providing insight not only into “who is there” but also “what they are doing” and “what they are producing”.

2.2 *Single-cell technologies and culturomics*

Single-cell technologies and culturomics complement multi-omics by directly attributing functional roles to UMs in fermented foods. Single-cell genomics isolates and sequences individual microbial genomes using tools such as microfluidics, flow cytometry, and whole-genome amplification. This approach is particularly powerful for low-abundance or unculturable taxa, allowing the recovery of nearly complete genomes and the identification of novel biosynthetic pathways. A representative example is the work of Zhang and colleagues, who employed flow cytometry and cell sorting to examine Baijiu stacked fermentation grains. They were able to pinpoint metabolically active bacterial genera such as *Herbaspirillum, Chitinophaga,* and *Ralstonia,* alongside fungal members like *Aspergillus* and *Pichia* [26], demonstrating how single-cell resolution uncovers hidden contributors to fermentation dynamics.

Culturomics, in parallel, expands the culturability of UMs by mimicking native ecological conditions under controlled laboratory settings. By systematically varying pH, redox potential, nutrient sources, salinity, and introducing co-culture systems, researchers can create micro-niches that enable the growth of otherwise unculturable microbes. When integrated with rapid identification techniques such as Matrix-Assisted Laser Desorption/Ionization Time-Of-Flight Mass Spectrometry (MALDI-TOF MS) and genome sequencing, culturomics substantially broadens the catalog of culturable taxa. For example, a previous study combined culturomics with amplicon-based metagenomics to analyze Baijiu pit muds, successfully isolating 215 prokaryotic strains, including 19 putative novel species [27]. Co-culture strategies, where UMs are cultivated alongside helper strains or within biofilm-like matrices, have further revealed metabolites and functional traits that mirror their activity *in situ* [28]. Together, these techniques validate the biosynthetic potential of UMs and, when integrated with multi-omics, accelerate the discovery of novel probiotics and postbiotics in fermented food ecosystems.

2.3 *Integration with AI and predictive modeling*

The massive datasets generated by multi-omics and single-cell studies necessitate advanced computational frameworks for meaningful interpretation. Artificial Intelligence (AI)-based modeling, including agent-based simulations and genome-scale metabolic reconstructions, has been proposed to predict microbial interactions, metabolic fluxes, and community dynamics [29, 30]. Agent-based modeling can simulate individual microbial behaviors and interactions within complex ecosystems, while genome-scale metabolic models reconstruct metabolic networks from genomic or metagenomic data, enabling prediction of nutritional requirements, syntrophic relationships, and functional outputs of unculturable taxa [29]. These frameworks provide conceptual avenues for rational prebiotic and synbiotic design, and for evaluating safety profiles and functional potential of UM-derived metabolites.

Concrete applications of AI in fermented foods are beginning to emerge. For example, machine-learning models trained on near-infrared spectroscopy and electronic-nose data have been used to classify flour types and predict volatile compounds in sourdough fermentation with high accuracy [31]. In kimchi, Fourier-transform near-infrared spectra analyzed by support vector machines and other algorithms successfully discriminated products by geographical origin, illustrating how AI can capture subtle biochemical and microbial signatures in traditional ferments [32]. Moreover, smart fermentation platforms integrating AI with biosensors and IoT technologies are being developed for real-time monitoring and control of microbial dynamics in artisanal fermentations [33]. These studies demonstrate that AI is not merely a futuristic concept but is already finding practical use in fermented food systems, setting the stage for its application to UMs as multi-omics datasets continue to expand.

3. Functional roles of UMs in fermentation

UMs though historically overlooked, are increasingly recognized for their functional contributions to fermentation ecosystems. Even in the absence of isolation and characterization via culture-based methods, metagenomic and metatranscriptomic analyses reveal that UMs actively participate in key metabolic pathways during fermentation. These organisms contribute to enzymatic transformations, the generation of bioactive compounds, and the modulation of sensory properties of fermented foods [34].

One of the primary roles of UMs in fermentation lies in enzymatic activity. Genes associated with carbohydrate-active enzymes, proteolysis, and lipolysis have been identified in uncultured taxa through metagenomics [35]. These enzymes are crucial in breaking down complex substrates into smaller molecules, influencing texture and nutrient availability [36]. Additionally, UMs have been implicated in the production of bioactive compounds such as bacteriocins [35], SCFAs, vitamins, and antioxidant peptides [5]. In several fermentation systems, functional gene clusters linked to antimicrobial activity and biosynthesis of secondary metabolites are abundant among uncultured taxa [35]. These metabolites may contribute to food safety, shelf stability, and potential probiotic effects, even if the originating organisms are not culturable [35]. The modulation of sensory characteristics—such as aroma, taste, and texture—is another important function mediated by UMs. Volatile compound profiles in fermented foods are shaped by microbial metabolic activity, and recent studies have shown that uncultured bacteria play significant roles in the synthesis of key esters, alcohols, and sulfur-containing compounds [8]. These compounds affect consumer perception and can vary depending on microbial community structure, including contributions from non-dominant or uncultured members.

In traditional Asian fermented foods, the functional roles of UMs are particularly significant due to the artisanal and open fermentation conditions. These conditions promote the development of complex microbial communities, which significantly influence the quality of the final product. In kimchi, for example, unclassified *Deferribacterales* and unclassified *Bacteria* dominate in the early stages of fermentation, but a significant portion of *Weissella*, *Leuconostoc*, and *Lactobacillus* species play active roles in acidification, vitamin synthesis, and aromatic compound production in the later stages [37]. These uncultured microbes interact dynamically with starter cultures and native microbes, influencing the fermentation trajectory and final product quality. In fish sauce (*nước mắm*), one of Southeast Asia's iconic fermented condiments, prolonged fermentation (6-12 months) and high salt content create a selective environment where only halotolerant and osmotolerant microorganisms can survive. While *Tetragenococcus halophilus* is often isolated as a dominant LAB, culture-independent methods have uncovered numerous uncultured halophiles and archaea potentially involved in amino acid deamination, biogenic amine degradation, and flavor compound synthesis [8, 38]. Moreover, these traditional systems exemplify interactions between indigenous microbes and introduced starter cultures, where UMs may modulate or be modulated by the metabolic outputs of dominant strains. This includes synergistic effects (e.g., cross-feeding on metabolites), antagonistic interactions (e.g., production of inhibitory compounds), and niche partitioning—all of which influence microbial succession, metabolite profiles, and product stability [39]. Understanding the functional roles of UMs in these contexts provides new insights into fermentation science and highlights the need to go beyond the “cultivable core” to fully harness the microbiological potential of fermented foods. Table 1 provides an overview of UMs in specific fermented foods and their roles.

Table 1. Uncultured microorganisms in fermented foods

Genera	Traditional fermented food/ Fermentation system	Properties of synthesis	Beneficial effects	References
		Dairy products		
Lactobacillus, *Streptococcus*, *Acetobacter*, and *Rothia*	Fermented milk products	Carbohydrate-active enzymes, secondary metabolite clusters, and bacteriocins	Influence food flavor, quality and/or safety	[35]
Actinobacteria, *Saccharomyces*, Enterobacteriaceae, and *Lactobacillus*	Kefir	Secondary metabolites (plipastatin, fengycin, and bacillaene), and also bacteriocins (helveticin and zoocin)	Against bacterial and fungal pathogens	[40]
Lactobacillus and *Lactococcus*	Kazakh artisanal fermented milk Ayran in Xinjiang, China	Proline iminopeptidase, dipeptidase, and proteases	Coagulation quality of milk	[41]
Lactobacillus, *Lactococcus*, *Streptococcus*, Enterococcaceae, Lactobacillaceae, Bifidobacteriaceae, and Acetobacteraceae	Traditional fermented milk products from Russia	Glycoside hydrolases, glycosyltransferases; lanthipeptides, unclassified bacteriocins, nonribosomal peptides, and polyketides	Synthesis of bioactive compounds	[42]
Enterococcus, *Lactococcus*, *Lactobacillus*, *Bifidobacterium*, *Acetobacter*, *Streptococcus*, and *Leuconostoc*	Indian ethnic fermented milk product	Short-chain fatty acids, antimicrobial peptides, amino acids, and vitamins	Health promoting functionalities	[5]
Firmicutes (Streptococcaceae, Lactobacillaceae) and Proteobacteria (Acetobacteraceae)	Naturally fermented milk products in Arunachal Pradesh and Sikkim states of India	—	—	[43]
Lactococcus, *Lactobacillus*, *Pseudoalteromonas*, and *Streptococcus*	Dairy and non-dairy fermented foods (mish, jibneh, zabadi, and pickles) in Saudi Arabia	Antimicrobial resistance, amino acid metabolism, carbohydrate metabolism, cofactors, and vitamin biosynthesis	Health effects	[44]
		Soy-based products		
Leuconostoc, *Staphylococcus*, *Tetragenococcus*, *Weissella*, *Wickerhamomyces*, *Zygosaccharomyces*, and *Aspergillus*	Soy sauce in China	Amino acids, alcohols, acids, and aldehydes	Degradation of nitrite/nitrate, flavor formation	[6]
Zygosaccharomyces, *Candida*, *Weissella*, *Pediococcus*, *Lactococcus*, *Acinetobacter*, *Pseudomonas*, *Bacillus*, *Halobacterium*, *Acinetobacter*, and *Halomonas*	Soy sauce fermentation	Ethanol accumulation, urea accumulation, citrulline, and arginine content	Reduce the accumulation of ethyl carbamate precursors in soy sauce	[7]
Firmicutes, Proteobacteria, Ascomycota, *Aspergillus*, and *Weissella*	Koji	Amino acids, volatile flavor compounds	Flavor formation during koji making	[45]
Aspergillus, *Bacillus*, *Enterococcus*, *Lactococcus*, *Rhizopus*, and *Penicillium*	Soy sauce production from black soybeans	Production of lactic acid	Development of soy sauce flavor	[46]
Staphylococcus, *Bacillus*, *Kurthia*, *Klebsiella*, *Aspergillus*, *Zygosaccharomyces*, *Trichosporon*, *Millerozyma*, and *Peronospora*	Soy sauce fermentation process	—	Improving the quality of soy sauce	[47]
Firmicutes, Proteobacteria, Bacteroidetes, and *Bacillus*	Pe poke, a naturally fermented soybean food of Myanmar	Amino acids, different vitamins, and enzymes	Health benefits, hygiene, and general safety in pe poke	[36]
Lactobacillus, *Pichia*, *Enterococcus*, *Bacillus*, and *Acetobacter*	Naturally fermented tofu whey	α-galactosidase activities, organic acids	Coagulator	[1]

Table 1. (cont.)

Genera	Traditional fermented food/ Fermentation system	Properties of synthesis	Beneficial effects	References
		Fish sauces		
Halanaerobium, *Halomonas*, *Tetragenococcus*, *Halococcus*, Candidatus, and *Fracribacter*	Chinese fish sauce (Yu-lu)	Volatile flavor compounds	Flavor formation	[8]
Lactobacillus, *Staphylococcus*, *Enterobacter*, etc.	Fish sauces	Flavor compounds	Flavor generation of fish sauce	[17]
Enterobacterales, Gammaproteobacteria, Fusobacteriaceae, *Halanaerobium*, *Staphylococcus*, *Lentibacillus*, Bacilli, and *Halomonas*	Malaysian fish sauce (bubu)	Volatile compounds	Quality control and fermentation monitoring	[48]
Lactobacillales, Enterococcaceae, *Tetragenococcus*, and *Staphylococcus*	Fermented sauce from Sumbawa, West Nusa Tenggara, Indonesia	—	—	[49]
		Vegetables/Olives		
Lactobacillus, Enterobacteriaceae	Fermented cabbage, carrot, and turnip	—	Influence microbial ecology, fermentation quality, and product safety	[50]
Rubrobacteraceae, Bifidobacteriaceae, and Ruminococcaceae	Fermented vegetable samples from China, Ireland, the UK, and Germany	Carbohydrate-active enzymes and antibiotic resistance genes	Fermented vegetable microbiota composition and functional potentials	[51]
Lactobacillus, *Celerinatimonas*, *Propionibacterium*, *Pseudomonas*, Pichiaceae, *Pichia*, *Ogataea*, and *Saccharomyces*	Fermented Kalamata black olives from Greece	—	—	[52]
Enterobacteriaceae, Lactobacillaceae, Phaffomycetaceae, and Pichiaceae	Fermented Greek Table olives	—	—	[53]

Note: Evidence type refers to the primary method of identification (omics, culturomics, or single-cell). Food property affected refers to the main functional or quality attribute influenced (e.g., flavor, amino-nitrogen, SCFA production, or safety). (—) No specific report available

4. UMs and human health: The missing link

Recent insights suggest that UMs are not merely passive inhabitants of fermented food microbiomes but may play critical roles in modulating human health. Despite the challenges associated with isolating and characterizing UMs, emerging evidence from multi-omics and functional studies indicates that their metabolites and cellular components actively influence the host immune system, support gut barrier integrity, and contribute to the stabilization of microbial ecosystems (Figure 2). Understanding these functional links highlights the potential of UMs as sources of next-generation probiotics and postbiotics.

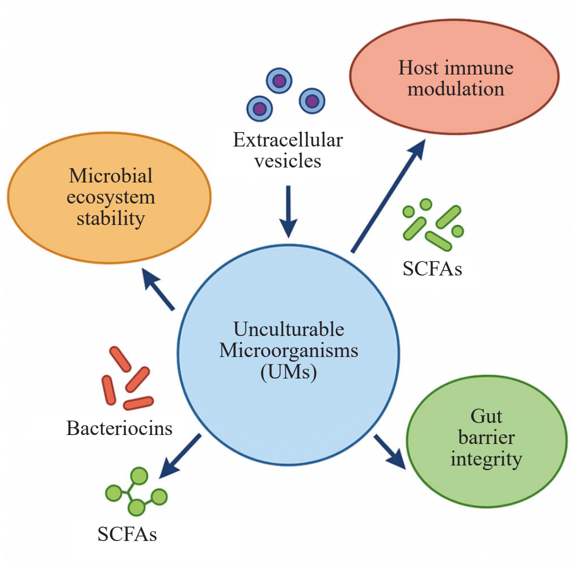

Figure 2. Interactions of UMs with microbial communities and host health, highlighting roles in immune modulation, gut barrier maintenance, and microbial ecosystem stabilization

4.1 *Modulating the host immune system*

One of the most significant contributions of UMs is their capacity to modulate the host immune response through the production of bioactive molecules. Studies have shown that metabolites such as SCFAs, EVs, and bacteriocins derived from microbial communities—including uncultured members—can directly interact with immune cells, influencing cytokine production and immune regulation [44, 54]. For example, EVs isolated from conditionally culturable lactic acid bacteria in fermented soybean pastes have demonstrated the ability to modulate dendritic cell maturation and promote anti-inflammatory cytokine profiles [54]. Similarly, SCFAs such as butyrate, acetate, and propionate—frequently associated with uncultured fermentative species—exhibit potent immunoregulatory effects by promoting regulatory T cell differentiation and suppressing pro-inflammatory signaling pathways [10]. UMs likely contribute to these effects either through direct metabolite production or by modulating the metabolic output of co-existing culturable microorganisms within the gut ecosystem. This immunomodulatory potential positions UMs as promising candidates for developing novel interventions targeting immune-mediated diseases.

4.2 *Supporting gut barrier integrity*

The integrity of the intestinal barrier is fundamental for maintaining host health and preventing translocation of pathogenic microorganisms and toxins. Several studies have indicated that metabolites and structural components derived from UMs may enhance gut epithelial function. SCFAs, particularly butyrate, produced or promoted by uncultured bacterial groups, strengthen tight junction integrity, stimulate mucus production, and promote epithelial cell renewal [55, 56]. These effects are crucial for preventing leaky gut syndrome and associated inflammatory disorders. Furthermore, non-viable microbial components such as peptidoglycans and cell wall fragments from UMs can interact with Pattern Recognition Receptors (PRRs) on intestinal epithelial cells, triggering protective immune responses that reinforce barrier function without inducing excessive inflammation [57, 58]. Given that many UMs exist in fermented foods consumed regularly across various cultures, their cumulative effects on gut barrier health may have been underestimated in traditional assessments focused solely on culturable probiotic strains.

4.3 *Stabilizing microbial ecosystems*

UMs also play essential roles in maintaining the stability and resilience of microbial communities within the gut. Fermented food-derived UMs contribute to microbial ecosystem balance through several mechanisms: cross-feeding interactions, competitive exclusion of pathogens, and modulation of environmental factors such as pH and redox potential [8, 39]. For instance, UMs may engage in syntrophic relationships where metabolic byproducts of one species serve as substrates for another, enhancing overall metabolic efficiency and community robustness [59]. These interactions create a more resilient microbiota capable of resisting perturbations such as antibiotic treatment or dietary shifts. Moreover, certain uncultured halophiles and archaea detected in traditional fermented products like fish sauce have been implicated in amino acid metabolism and biogenic amine degradation, processes that help maintain microbial homeostasis and food safety [8, 38, 60]. By contributing to ecosystem stability and diversity, UMs indirectly support host health, emphasizing the importance of considering the entire microbiome—including unculturable members—when designing functional foods or therapeutic interventions.

5. Applications of UMs in functional foods and therapeutics

With the advancement of microbiome science, UMs are increasingly recognized not merely as passive members of microbial consortia, but as potential functional agents in health and nutrition. These applications center around three emerging domains: next-generation probiotics, postbiotic production, and synbiotic formulation (Figure 3).

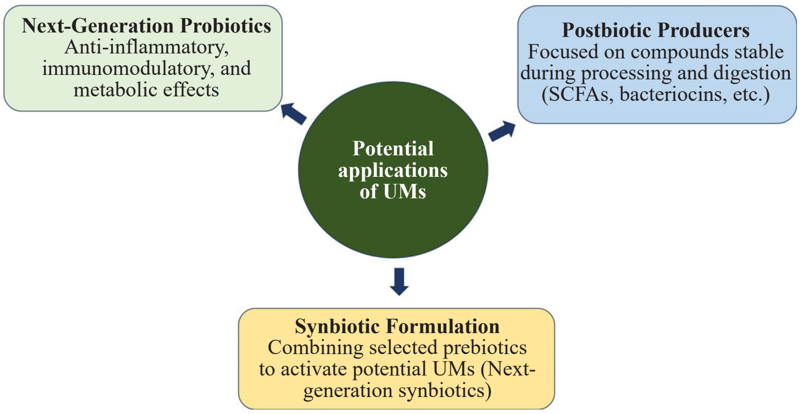

Figure 3. Functional applications of UMs in fermented foods, including their use as next-generation probiotics, postbiotic producers, and targets for synbiotic formulations

5.1 *As next-generation probiotics*

Traditional probiotics—such as *Lactobacillus* and *Bifidobacterium*—have demonstrated beneficial effects [61], but they represent only a small fraction of the gut microbiota. Recent studies have turned attention toward UMs that are abundant, functionally active, yet recalcitrant to classical cultivation techniques, many of which exert immunomodulatory or metabolic benefits [10]. Two widely studied examples are *Akkermansia muciniphila* and *Faecalibacterium prausnitzii*. These species were previously considered difficult to culture but are now regarded as next-generation probiotics due to their anti-inflammatory and metabolic regulatory properties. *A. muciniphila* enhances mucin turnover, tight junction integrity, and improves glucose homeostasis and lipid metabolism in animal and human models [55, 62]. *F. prausnitzii* produces butyrate and anti-inflammatory peptides, reducing colonic inflammation in patients with Crohn's disease and ulcerative colitis [63, 64]. Although not all UMs can be directly cultivated, their therapeutic potential can still be accessed via their functional components or through engineered delivery systems, such as encapsulated lysates or synthetic microbial consortia [65].

5.2 *As postbiotic producers*

UMs, even when non-viable, may act as producers of postbiotics—bioactive compounds derived from microbial metabolism or structural components. Postbiotics offer several advantages: they are stable, easier to standardize, and do not require viability, which is critical for food applications involving high heat or long shelf lives [66]. Compounds such as SCFAs, bacteriocins, peptidoglycans, exopolysaccharides, and EVs derived from UMs play essential roles in host immunity, gut barrier integrity, and pathogen suppression [44]. In particular, EVs from uncultured LAB in representative fermented foods have been shown to carry immunomodulatory molecules that affect dendritic cell maturation and cytokine profiles [54]. Inactivating UMs through methods like heat treatment or sonication can preserve structural components such as cell wall fragments and DNA motifs. These preserved structures can interact with host Pattern Recognition Receptors (PRRs), eliciting beneficial effects similar to those of viable probiotics, while offering enhanced safety and shelf stability [57, 58, 67].

5.3 *Synbiotic formulation*

Another promising strategy is the development of next-generation synbiotics, which involve pairing prebiotics with specific UMs or their functional guilds to promote health. Unlike classical synbiotics—which often combine generic oligosaccharides with culturable strains—next-gen synbiotics emphasize selective substrate utilization by specific UMs, supporting their growth and functionality *in situ* [68]. For instance, certain arabinoxylans, resistant starches, and flavonoid-rich fibers selectively stimulate the activity of butyrate-producing UMs, including *Roseburia* spp. and *Eubacterium hallii*, which are otherwise challenging to maintain in the gut [69, 70]. Metagenomic-guided prebiotic design allows for matching substrate specificity to UM metabolic profiles. These personalized synbiotic approaches hold strong potential in the context of precision nutrition, where host genotype, microbiome composition, and lifestyle factors guide dietary interventions. In fermented food development, incorporating tailored prebiotics into traditional substrates may enhance the growth of beneficial UMs, enabling designer foods with specific health claims [71].

6. Industrial innovations: From UMs to products

The functional potential of UMs has catalyzed innovative strategies for their exploitation in the food, nutraceutical, and biopharmaceutical industries. Given the inherent challenges of cultivating UMs using conventional methods, novel technological platforms have been developed to harness their benefits through metabolite extraction, synthetic biology, and advanced *in vitro* modeling systems. Figure 4 highlights how UMs, though elusive in nature, are increasingly explored through predictive tools such as gut-on-chip simulations and computational modeling. These approaches bridge discovery to downstream applications, including synthetic biology and engineered postbiotic products.

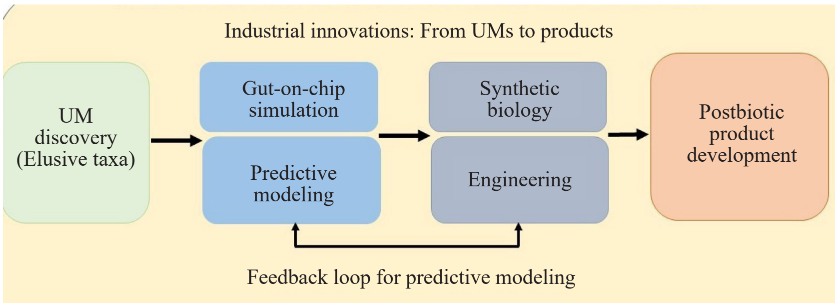

Figure 4. Industrial innovations: from the elusive discovery of UMs to predictive simulations and engineering pathways for postbiotic product generation

6.1 *Gut-on-chip simulation for UM evaluation*

Gut-on-chip systems replicate the dynamic human gastrointestinal environment using microfluidic devices, integrating mechanical forces (e.g., peristalsis), fluid flow, and co-cultures of intestinal epithelial cells with microbiota [72, 73]. Such platforms enable the real-time investigation of UM-derived metabolites and structural components on gut health parameters, including barrier integrity, immune signaling, and microbial community dynamics. Importantly, gut-on-chip models circumvent the need for direct cultivation of UMs by focusing on their functional outputs, offering a powerful tool for screening postbiotic candidates, evaluating synbiotic efficacy, and studying host-microbe interactions in a controlled, reproducible environment.

6.2 *Synthetic biology: Engineering UM functions*

Synthetic biology provides an alternative route to leverage UM functionality by transferring genes or biosynthetic pathways from UMs into culturable, well-characterized host strains. Using techniques such as modular genetic circuit design, CRISPR-mediated genome editing, and heterologous expression systems, key enzymes, bacteriocins, or bioactive metabolite pathways from UMs can be produced at an industrial scale [74, 75]. For example, bacteriocin genes identified in metagenomic datasets of fermented foods have been successfully expressed in *Lactobacillus plantarum*, enhancing its postbiotic production capacity [76]. Such synthetic biology strategies bypass the barriers of cultivation while enabling precise control over product yield, activity, and quality—critical factors for industrial application. Moreover, the integration of synthetic biology with predictive modeling allows the rational design of synthetic microbial consortia tailored for specific health outcomes, utilizing functional traits originally derived from UMs.

6.3 *Postbiotic product development*

Recognizing the logistical and regulatory challenges associated with live microorganisms, there is a growing shift toward developing non-viable, microbiome-derived products based on UM metabolites and structural components. Postbiotic formulations—including purified SCFA blends, EVs, cell-free supernatants, and heat-inactivated microbial lysates—offer enhanced stability, safety, and standardization [44, 66]. For example, fermented soybean-derived postbiotics enriched with EVs from uncultured *Bacillus* spp. have demonstrated immunonutritional benefits and potential as functional food ingredients [77]. In pharmaceutical applications, targeted delivery systems such as pH-sensitive capsules and lipid nanoparticles are being explored to ensure the bioavailability and efficacy of UM-derived postbiotic compounds in the gastrointestinal tract [78, 79]. The industrial scalability of postbiotic production, coupled with favorable regulatory prospects for non-viable products, positions UM-based postbiotics as a promising frontier in functional food innovation and microbiome-based therapy development.

7. Regulatory and safety considerations

The application of UMs and their metabolites in functional foods and therapeutics introduces a regulatory grey zone, primarily due to the inability to cultivate and characterize them using traditional methods. This raises challenges for safety assessment, standardization, and regulatory approval, particularly when products are intended for ingestion or therapeutic use.

7.1 *Safety challenges due to lack of cultivation*

Conventional microbial safety evaluations, such as those required for Qualified Presumption of Safety (QPS) by the European Food Safety Authority (EFSA) or Generally Recognized as Safe (GRAS) status by the U.S. FDA, rely heavily on the availability of a pure culture, comprehensive genomic sequencing, and historical data on pathogenicity, toxin production, and antibiotic resistance profiles [80, 81]. These criteria are difficult to apply to UMs, which by definition cannot be cultured under standard laboratory conditions. In the absence of culture-based data, UMs cannot undergo traditional taxonomy-based risk assessment. Their roles within microbial communities remain context-dependent, and their genetic content is often inferred solely through metagenomic assemblies, which may lack strain-level resolution [82].

7.2 *Metabolite-centered safety assessment*

Given these limitations, a promising shift is underway toward evaluating the safety of UMs-derived products based on their metabolic outputs, especially when these products are postbiotic in nature. This approach focuses on measurable and stable compounds such as SCFAs, bacteriocins, peptidoglycans, cell-free supernatants, and EVs, which can be chemically characterized and quantified. As evidenced by non-viable cells or cell fragments from representative next-generation UMs with strong immunomodulatory effects, which have shown potential in reducing inflammation without needing viable cells, suggesting a metabolite-oriented regulatory model [56, 83, 84]. This aligns with recent EFSA opinions, which recognize that safety can be demonstrated at the compound level, especially when no viable cells are present [85].

7.3 *Toward specific regulatory frameworks for UMs-derived products*

To bridge this gap, experts have proposed expanding existing frameworks or establishing new legal categories specifically for UMs-based postbiotics. First, the integration of postbiotic-specific criteria into EFSA's QPS guidelines could standardize safety assessments. This would include requirements such as metabolite profiling, *in vitro* and *in vivo* toxicity testing, and functionality assays to confirm immunomodulatory or gut barrier-enhancing effects. Second, a shift from taxonomic identity to function-based classification is warranted—defining "functional postbiotic units" based on bioactivity rather than microbial origin may better capture the therapeutic value of non-viable cells or their metabolic products. Finally, for UMs, a dedicated risk evaluation pipeline should be established. This pipeline would combine high-resolution metagenomic data with biosynthetic pathway annotation (e.g., screening for absence of virulence factors or toxin genes), and metabolomic profiling to assess bioactive compounds for safety and efficacy.

8. Conclusion and future directions

UMs are increasingly recognized as key players in fermented food ecosystems, contributing to enzymatic transformations, bioactive metabolite production, flavor development, and microbial stability. Once overlooked due to cultivation barriers, UMs are now positioned at the center of next-generation microbiome-based strategies. Postbiotics derived from UMs—such as SCFAs, EVs, and other metabolites—offer attractive alternatives to conventional probiotics by combining safety, stability, and targeted functionality. Technological advances are gradually transforming UMs from hidden taxa into actionable resources. Multi-omics approaches reveal their diversity and metabolic capacity, while single-cell genomics and culturomics help bridge genome inference and cultivation. Gut-on-chip platforms further allow controlled testing of UM-derived metabolites and host responses, and synthetic biology enables the transfer of UM pathways into culturable hosts for scalable production. Together, these innovations build the foundation for food, nutraceutical, and pharmaceutical applications.

Future studies should prioritize *in vivo* validation of UM-derived metabolites and postbiotics using gut-on-chip models and animal trials to confirm efficacy and safety. In parallel, longitudinal metagenomic and metabolomic surveys of traditional fermented foods are needed to establish comprehensive UM reference databases, particularly from underexplored regions such as Southeast Asia. On the industrial side, scaling up precision fermentation and synthetic biology approaches will be essential for producing UM-derived compounds at commercial levels. Finally, collaboration with regulatory agencies should aim to develop standardized metabolite-centered safety frameworks, ensuring that UM-based innovations can be translated effectively into functional foods and therapeutic applications.

Acknowledgments

The authors sincerely thank the Science and Technology Project of Can Tho City, Vietnam, for funding this research.

Authorship contribution

Phu-Tho Nguyen: Conceptualization, Literature search, Writing-original draft preparation. Huu-Thanh Nguyen: Writing-review and editing.

Conflict of interest

The authors declare no competing interests.

References

[1] Fei Y, Li L, Chen L, Zheng Y, Yu B. High-throughput sequencing and culture-based approaches to analyze microbial diversity associated with chemical changes in naturally fermented tofu whey, a traditional Chinese *tofu*-coagulant. *Food Microbiology*. 2018; 76: 69-77. Available from: https://doi.org/10.1016/j.fm.2018.04.004.

[2] Hernández-Velázquez R, Flörl L, Lavrinienko A, Sebechlebská Z, Merk L, Greppi A, et al. The future is fermented: Microbial biodiversity of fermented foods is a critical resource for food innovation and human health. *Trends in Food Science & Technology*. 2024; 150: 104569. Available from: https://doi.org/10.1016/j.tifs.2024.104569.

[3] Selim S, Harun-Ur-Rashid M, Jahan I, Mostafa EM. Culture-independent molecular techniques for bacterial detection in bivalves. *Egyptian Journal of Aquatic Research*. 2024; 50(4): 585-600. Available from: https://doi.org/10.1016/j.ejar.2024.11.002.

[4] Wang J, Hao S, Ren Q. Uncultured microorganisms and their functions in the fermentation systems of traditional Chinese fermented foods. *Foods*. 2023; 12(14): 2691. Available from: https://doi.org/10.3390/foods12142691.

[5] Shangpliang HNJ, Tamang JP. Metagenome-assembled genomes for biomarkers of bio-functionalities in *Laal dahi*, an Indian ethnic fermented milk product. *International Journal of Food Microbiology*. 2023; 402: 110300. Available from: https://doi.org/10.1016/j.ijfoodmicro.2023.110300.

[6] Liu H, Chen Z, Ma M, Zhang Y, Li D, Lu J, et al. Metagenomic analysis of the relationship between microorganisms and flavor development during soy sauce fermentation. *Food Bioscience*. 2023; 56: 103193. Available from: https://doi.org/10.1016/j.fbio.2023.103193.

[7] Liu X, Bai W, Zhao W, Qian M, Dong H. Correlation analysis of microbial communities and precursor substances of ethyl carbamate (EC) during soy sauce fermentation. *LWT-Food Science and Technology*. 2021; 152: 112288. Available from: https://doi.org/10.1016/j.lwt.2021.112288.

[8] Liu Y, Pan T, Tang Y, Zhuang Y, Liu Z, Li P, et al. Proteomic analysis of rice subjected to low light stress and overexpression of *OsGAPB* increases the stress tolerance. *Rice*. 2020; 13(1): 30. Available from: https://doi.org/10.1186/s12284-020-00390-8.

[9] Thorakkattu P, Khanashyam AC, Shah K, Sajith Babu K, Shanker MA, Deliephan A, et al. Postbiotics: Current trends in food and pharmaceutical industry. *Foods*. 2022; 11(19): 3094. Available from: https://doi.org/10.3390/foods11193094.

[10] Lu S, Wang C, Ma J, Wang Y. Metabolic mediators: Microbial-derived metabolites as key regulators of anti-tumor immunity, immunotherapy, and chemotherapy. *Frontiers in Immunology*. 2024; 15: 1456030. Available from: https://doi.org/10.3389/fimmu.2024.1456030.

[11] Berdy B, Spoering AL, Ling LL, Epstein SS. In situ cultivation of previously uncultivable microorganisms using the ichip. *Nature Protocols*. 2017; 12(10): 2232-2242. Available from: https://doi.org/10.1038/nprot.2017.074.

[12] Doud DFR, Woyke T. Novel approaches in function-driven single-cell genomics. *FEMS Microbiology Reviews*. 2017; 41(4): 538-548. Available from: https://doi.org/10.1093/femsre/fux009.

[13] Wu Y, Zhuang J, Song Y, Gao X, Chu J, Han S. Advances in single-cell sequencing technology in microbiome research. *Genes & Diseases*. 2024; 11(4): 101129. Available from: https://doi.org/10.1016/j.gendis.2023.101129.

[14] Wang Y, Wu Y, Li C, Zhao Y, Xiang H, Li L, et al. Genome-resolved metaproteomic analysis of microbiota and metabolic pathways involved in taste formation during Chinese traditional fish sauce (*Yu-lu*) fermentation. *Frontiers in Nutrition*. 2022; 9: 851895. Available from: https://doi.org/10.3389/fnut.2022.851895.

[15] Das R, Tamang B, Najar IN, Thakur N, Mondal K. First report on metagenomics and their predictive functional analysis of fermented bamboo shoot food of Tripura, North East India. *Frontiers in Microbiology*. 2023; 14: 1158411. Available from: https://doi.org/10.3389/fmicb.2023.1158411.

[16] Zhang J, Wang X, Huo D, Li W, Hu Q, Xu C, et al. Metagenomic approach reveals microbial diversity and predictive microbial metabolic pathways in *Yucha*, a traditional Li fermented food. *Scientific Reports*. 2016; 6(1): 32524. Available from: https://doi.org/10.1038/srep32524.

[17] Han J, Kong T, Jiang J, Zhao X, Zhao X, Li P, et al. Characteristic flavor metabolic network of fish sauce microbiota with different fermentation processes based on metagenomics. *Frontiers in Nutrition*. 2023; 10: 1121310. Available from: https://doi.org/10.3389/fnut.2023.1121310.

[18] Pan Y, Wang Y, Hao W, Duan C, Wang S, Wei J, et al. Metatranscriptomics unravel composition, drivers, and functions of the active microorganisms in light-flavor liquor fermentation. *Microbiology Spectrum*. 2022; 10(3): e02151-02121. Available from: https://doi.org/10.1128/spectrum.02151-21.

[19] Xiao Y, Huang T, Yazhou X, Peng Z, Liu Z, Guan Q, et al. Metatranscriptomics reveals the gene functions and metabolic properties of the major microbial community during Chinese Sichuan *Paocai* fermentation. *Food Microbiology*. 2020; 98: 103573. Available from: https://doi.org/10.1016/j.fm.2020.103573.

[20] Jung JY, Lee SH, Jin HM, Hahn Y, Madsen EL, Jeon CO. Metatranscriptomic analysis of lactic acid bacterial gene expression during kimchi fermentation. *International Journal of Food Microbiology*. 2013; 163(2): 171-179. Available from: https://doi.org/10.1016/j.ijfoodmicro.2013.02.022.

[21] An F, Li M, Zhao Y, Zhang Y, Mu D, Hu X, et al. Metatranscriptome-based investigation of flavor-producing core microbiota in different fermentation stages of *dajiang*, a traditional fermented soybean paste of Northeast China. *Food Chemistry*. 2020; 343: 128509. Available from: https://doi.org/10.1016/j.foodchem.2020.128509.

[22] Yang L, Fan W, Xu Y. Metaproteomics insights into traditional fermented foods and beverages. *Comprehensive Reviews in Food Science and Food Safety*. 2020; 19(5): 2506-2529. Available from: https://doi.org/10.1111/1541-4337.12601.

[23] Rizo J, Guillén D, Díaz G, Wacher C, Encarnación S, Sánchez S, et al. Metaproteomic insights into the microbial community in *Pozol*. *Frontiers in Nutrition*. 2021; 8: 714814. Available from: https://doi.org/10.3389/fnut.2021.714814.

[24] Rafique N, Jan SY, Dar AH, Dash KK, Sarkar A, Shams R, et al. Promising bioactivities of postbiotics: A comprehensive review. *Journal of Agriculture and Food Research*. 2023; 14: 100708. Available from: https://doi.org/10.1016/j.jafr.2023.100708.

[25] Chung YB, Park SJ, Choi Y-J, Yun Y-R, Lee M-A, Park SH, et al. Metabolic shift during fermentation in kimchi according to capsaicinoid concentration. *Heliyon*. 2024; 10(2): e24441. Available from: https://doi.org/10.1016/j.heliyon.2024.e24441.

[26] Zhang Z, Wei Y, Peng Z, Du P, Du X, Zuo G, et al. Exploration of microbiome diversity of stacked fermented grains by flow cytometry and cell sorting. *Frontiers in Microbiology*. 2023; 14: 1160552. Available from: https://doi.org/10.3389/fmicb.2023.1160552.

[27] Xu J, Sun L, Xing X, Sun Z, Gu H, Lu X, et al. Culturing bacteria from fermentation pit muds of *Baijiu* with culturomics and amplicon-based metagenomic approaches. *Frontiers in Microbiology*. 2020; 11: 01223. Available from: https://doi.org/10.3389/fmicb.2020.01223.

[28] Fan Y, Huang X, Chen J, Han B. Formation of a mixed-species biofilm is a survival strategy for unculturable lactic acid bacteria and *Saccharomyces cerevisiae* in *Daqu*, a Chinese traditional fermentation starter. *Frontiers in Microbiology*. 2020; 11: 00138. Available from: https://doi.org/10.3389/fmicb.2020.00138.

[29] Choi Y-M, Lee YQ, Song H-S, Lee D-Y. Genome scale metabolic models and analysis for evaluating probiotic potentials. *Biochemical Society Transactions*. 2020; 48(4): 1309-1321. Available from: https://doi.org/10.1042/BST20190668.

[30] Nagarajan K, Ni C, Lu T. Agent-based modeling of microbial communities. *ACS Synthetic Biology*. 2022; 11(11): 3564-3574. Available from: https://doi.org/10.1021/acssynbio.2c00411.

[31] Gonzalez Viejo C, Harris NM, Fuentes S. Quality traits of sourdough bread obtained by novel digital technologies and machine learning modelling. *Fermentation*. 2022; 8(10): 516. Available from: https://doi.org/10.3390/fermentation8100516.

[32] Kim S-Y, Ha J-H. Rapid determination of the geographical origin of kimchi by Fourier transform near-infrared spectroscopy coupled with chemometric techniques. *Scientific Reports*. 2024; 14(1): 24581. Available from: https://doi.org/10.1038/s41598-024-74662-4.

[33] Yee CS, Zahia-Azizan NA, Abd Rahim MH, Mohd Zaini NA, Raja-Razali RB, Ushidee-Radzi MA, et al. Smart fermentation technologies: Microbial process control in traditional fermented foods. *Fermentation*. 2025; 11(6): 323. Available from: https://doi.org/10.3390/fermentation11060323.

[34] Zhao X, Xiang F, Tang F, Cai W, Guo Z, Hou Q, et al. Bacterial communities and prediction of microbial metabolic

pathway in rice wine *Koji* from different regions in China. *Frontiers in Microbiology*. 2022; 12: 748779. Available from: https://doi.org/10.3389/fmicb.2021.748779.

[35] You L, Chengcong Y, Jin H, Kwok L-Y, Sun Z, Zhang H. Metagenomic features of traditional fermented milk products. *LWT*. 2022; 155: 112945. Available from: https://doi.org/10.1016/j.lwt.2021.112945.

[36] Tamang JP, Kharnaior P, Pariyar P, Thapa N, Lar N, Win KS, et al. Shotgun sequence-based metataxonomic and predictive functional profiles of *Pe poke*, a naturally fermented soybean food of Myanmar. *PLOS One*. 2021; 16(12): e0260777. Available from: https://doi.org/10.1371/journal.pone.0260777.

[37] Jung JY, Lee SH, Kim JM, Park MS, Bae J-W, Hahn Y, et al. Metagenomic analysis of kimchi, a traditional Korean fermented food. *Applied and Environmental Microbiology*. 2011; 77(7): 2264-2274. Available from: https://doi.org/10.1128/AEM.02157-10.

[38] Fukui Y, Yoshida M, Shozen K-I, Funatsu Y, Takano T, Oikawa H, et al. Bacterial communities in fish sauce mash using culture-dependent and-independent methods. *The Journal of General and Applied Microbiology*. 2012; 58(4): 273-281. Available from: https://doi.org/10.2323/jgam.58.273.

[39] Hu G, Wang Y, Chen J, Du G, Fang F. Synergistic fermentation with functional bacteria for production of salt-reduced soy sauce with enhanced aroma and saltiness. *Food Bioscience*. 2024; 57: 103459. Available from: https://doi.org/10.1016/j.fbio.2023.103459.

[40] Tenorio-Salgado S, Castelán-Sánchez HG, Dávila-Ramos S, Huerta-Saquero A, Rodríguez-Morales S, Merino-Pérez E, et al. Metagenomic analysis and antimicrobial activity of two fermented milk kefir samples. *MicrobiologyOpen*. 2021; 10(2): e1183. Available from: https://doi.org/10.1002/mbo3.1183.

[41] Kuerman M, Wang R, Zhou Y, Tian X, Cui Q, Yi H, et al. Metagenomic insights into bacterial communities and functional genes associated with texture characteristics of Kazakh artisanal fermented milk *Ayran* in Xinjiang, China. *Food Research International*. 2023; 164: 112414. Available from: https://doi.org/10.1016/j.foodres.2022.112414.

[42] Elcheninov AG, Zayulina KS, Klyukina AA, Kremneva MK, Kublanov IV, Kochetkova TV. Metagenomic insights into the taxonomic and functional features of traditional fermented milk products from Russia. *Microorganisms*. 2023; 12(1): 16. Available from: https://doi.org/10.3390/microorganisms12010016.

[43] Shangpliang HNJ, Rai R, Keisam S, Jeyaram K, Tamang JP. Bacterial community in naturally fermented milk products of Arunachal Pradesh and Sikkim of India analysed by high-throughput amplicon sequencing. *Scientific Reports*. 2018; 8(1): 1532. Available from: https://doi.org/10.1038/s41598-018-19524-6.

[44] Yasir M, Alkhaldy AA, Soliman SA, Turkistani SA, Azhar EI. Metagenomic insights into the microbiome and resistance genes of traditional fermented foods in Arabia. *Foods*. 2023; 12(18): 3342. Available from: https://doi.org/10.3390/foods12183342.

[45] Tan G, Hu M, Li X, Li X, Pan Z, Li M, et al. Microbial community and metabolite dynamics during soy sauce *Koji* making. *Frontiers in Microbiology*. 2022; 13: 841529. Available from: https://doi.org/10.3389/fmicb.2022.841529.

[46] Nguyen NTH, Huang MB, Liu FY, Huang W-L, Tran H-T, Hsu T-W, et al. Deciphering microbial community dynamics along the fermentation course of soy sauce under different temperatures using metagenomic analysis. *Bioscience of Microbiota, Food and Health*. 2023; 42(2): 104-113. Available from: https://doi.org/10.12938/bmfh.2022-012.

[47] Wei Q, Wang H, Chen Z, Lv Z, Xie Y, Lu F. Profiling of dynamic changes in the microbial community during the soy sauce fermentation process. *Applied Microbiology and Biotechnology*. 2013; 97(20): 9111-9119. Available from: https://doi.org/10.1007/s00253-013-5146-9.

[48] Lestari SD, Hussin ASM, Mustafa S, Sew YS, Gan HM, Hashim AM, et al. Bacterial community structure, predicted metabolic activities, and formation of volatile compounds attributed to Malaysian fish sauce flavour. *Food Chemistry*. 2023; 426: 136568. Available from: https://doi.org/10.1016/j.foodchem.2023.136568.

[49] Manguntungi B, Saputri D, Mustopa A, Ekawati N, Afgani C, Sari AP, et al. Metagenomic analysis and biodiversity of lactic acid bacteria (LAB) on *masin* (fermented sauce) from Sumbawa, West Nusa Tenggara, Indonesia. *Biodiversitas Journal of Biological Diversity*. 2020; 21: 1787-1793. Available from: https://doi.org/10.13057/biodiv/d210752.

[50] Cantuti Gendre J, Le Marrec C, Chaillou S, Omhover-Fougy L, Landaud S, Dugat-Bony E. Exploring viral diversity in fermented vegetables through viral metagenomics. *Food Microbiology*. 2025; 128: 104733. Available from: https://doi.org/10.1016/j.fm.2025.104733.

[51] Liu Y, Yu L, Tian F, Chen W, Zhai Q. Meta-analysis of microbiomes reveals metagenomic features of fermented vegetables. *Food Research International*. 2023; 173: 113248. Available from: https://doi.org/10.1016/j.foodres.2023.113248.

[52] Kazou M, Tzamourani A, Panagou EZ, Tsakalidou E. Unraveling the microbiota of natural black cv. *Kalamata* fermented olives through 16S and ITS metataxonomic analysis. *Microorganisms*. 2020; 8(5): 672. Available from: https://doi.org/10.3390/microorganisms8050672.

[53] Argyri K, Doulgeraki AI, Manthou E, Grounta A, Argyri AA, Nychas G-JE, et al. Microbial diversity of fermented Greek table olives of Halkidiki and Konservolia varieties from different regions as revealed by metagenomic analysis. *Microorganisms*. 2020; 8: 1241. Available from: https://doi.org/10.3390/microorganisms8081241.

[54] Kim S-H, Lee J, Kim E, Reaney M, Shim Y, Chung M. Immunomodulatory activity of extracellular vesicles of kimchi-derived lactic acid bacteria (*Leuconostoc mesenteroides*, *Latilactobacillus curvatus*, and *Lactiplantibacillus plantarum*). *Foods*. 2022; 11(3): 313. Available from: https://doi.org/10.3390/foods11030313.

[55] Niu H, Zhou M, Zogona D, Xing Z, Wu T, Chen R, et al. *Akkermansia muciniphila*: A potential candidate for ameliorating metabolic diseases. *Frontiers in Immunology*. 2024; 15: 1370658. Available from: https://doi.org/10.3389/fimmu.2024.1370658.

[56] Wang Y, Li L, Chen S, Yu Z, Gao X, Peng X, et al. *Faecalibacterium prausnitzii*-derived extracellular vesicles alleviate chronic colitis-related intestinal fibrosis by macrophage metabolic reprogramming. *Pharmacological Research*. 2024; 206: 107277. Available from: https://doi.org/10.1016/j.phrs.2024.107277.

[57] Lin W-Y, Kuo Y-W, Chen C-W, Huang Y-F, Hsu C-H, Lin J-H, et al. Viable and heat-killed probiotic strains improve oral immunity by elevating the IgA concentration in the oral mucosa. *Current Microbiology*. 2021; 78(9): 3541-3549. Available from: https://doi.org/10.1007/s00284-021-02569-8.

[58] Maehata H, Arai S, Iwabuchi N, Abe F. Immuno-modulation by heat-killed *Lacticaseibacillus paracasei* MCC1849 and its application to food products. *International Journal of Immunopathology and Pharmacology*. 2021; 35: 20587384211008291. Available from: https://doi.org/10.1177/20587384211008291.

[59] Cheng T, Zhang T, Zhang P, He X, Sadiq FA, Li J, et al. The complex world of kefir: Structural insights and symbiotic relationships. *Comprehensive Reviews in Food Science and Food Safety*. 2024; 23(4): e13364. Available from: https://doi.org/10.1111/1541-4337.13364.

[60] Jeyaram K, Lahti L, Tims S, Heilig HGHJ, van Gelder AH, de Vos WM, et al. Fermented foods affect the seasonal stability of gut bacteria in an Indian rural population. *Nature Communications*. 2025; 16(1): 771. Available from: https://doi.org/10.1038/s41467-025-56014-6.

[61] Sarita B, Samadhan D, Hassan MZ, Kovaleva EG. A comprehensive review of probiotics and human health-current prospective and applications. *Frontiers in Microbiology*. 2025; 15: 1487641. Available from: https://doi.org/10.3389/fmicb.2024.1487641.

[62] Everard A, Belzer C, Geurts L, Ouwerkerk JP, Druart C, Bindels LB, et al. Cross-talk between *Akkermansia muciniphila* and intestinal epithelium controls diet-induced obesity. *Proceedings of the National Academy of Sciences*. 2013; 110(22): 9066-9071. Available from: https://doi.org/10.1073/pnas.1219451110.

[63] Cao Y, Shen J, Ran ZH. Association between *Faecalibacterium prausnitzii* reduction and inflammatory bowel disease: a meta-analysis and systematic review of the literature. *Gastroenterology Research and Practice*. 2014; 2014(1): 872725. Available from: https://doi.org/10.1155/2014/872725.

[64] Quévrain E, Maubert MA, Michon C, Chain F, Marquant R, Tailhades J, et al. Identification of an anti-inflammatory protein from *Faecalibacterium prausnitzii*, a commensal bacterium deficient in Crohn's disease. *Gut*. 2016; 65(3): 415. Available from: https://doi.org/10.1136/gutjnl-2014-307649.

[65] Li L, Yang C, Ma B, Lu S, Liu J, Pan Y, et al. Hydrogel-encapsulated engineered microbial consortium as a photoautotrophic "living material" for promoting skin wound healing. *ACS Applied Materials & Interfaces*. 2023; 15(5): 6536-6547. Available from: https://doi.org/10.1021/acsami.2c20399.

[66] Kumar A, Green KM, Rawat M. A comprehensive overview of postbiotics with a special focus on discovery techniques and clinical applications. *Foods*. 2024; 13(18): 2937. Available from: https://doi.org/10.3390/foods13182937.

[67] Karbowiak M, Gałek M, Szydłowska A, Zielińska D. The influence of the degree of thermal inactivation of probiotic lactic acid bacteria and their postbiotics on aggregation and adhesion inhibition of selected pathogens. *Pathogens*. 2022; 11: 1260. Available from: https://doi.org/10.3390/pathogens11111260.

[68] Fuhren J, Rösch C, Ten Napel M, Schols HA, Kleerebezem M. Synbiotic matchmaking in *Lactobacillus plantarum*: substrate screening and gene-trait matching to characterize strain-specific carbohydrate utilization. *Applied and Environmental Microbiology*. 2020; 86(18): e01081-01020. Available from: https://doi.org/10.1128/AEM.01081-20.

[69] Schwiertz A, Lehmann U, Jacobasch G, Blaut M. Influence of resistant starch on the SCFA production and cell counts of butyrate-producing *Eubacterium* spp. in the human intestine. *Journal of Applied Microbiology*. 2002;

93(1): 157-162. Available from: https://doi.org/10.1046/j.1365-2672.2002.01679.x.

[70] Liu J, Liu F, Arıoğlu-Tuncil S, Xie Z, Fu X, Huang Q, et al. *In vitro* faecal fermentation outcomes and microbiota shifts of resistant starch spherulites. *International Journal of Food Science & Technology*. 2022; 57(5): 2782-2792. Available from: https://doi.org/10.1111/ijfs.15237.

[71] Oliveira FLD, Arruda TYP, Morzelle MC, Pereira APA, Casarotti SN. Fruit by-products as potential prebiotics and promising functional ingredients to produce fermented milk. *Food Research International*. 2022; 161: 111841. Available from: https://doi.org/10.1016/j.foodres.2022.111841.

[72] Kim HJ, Li H, Collins JJ, Ingber DE. Contributions of microbiome and mechanical deformation to intestinal bacterial overgrowth and inflammation in a human gut-on-a-chip. *Proceedings of the National Academy of Sciences*. 2016; 113(1): E7-E15. Available from: https://doi.org/10.1073/pnas.1522193112.

[73] Jalili-Firoozinezhad S, Gazzaniga FS, Calamari EL, Camacho DM, Fadel CW, Nestor B, et al. Complex human gut microbiome cultured in anaerobic human intestine chips. *bioRxiv*. 2018; 421404. Available from: https://doi.org/10.1101/421404.

[74] Zhang Y, Chen M, Bruner SD, Ding Y. Heterologous production of microbial ribosomally synthesized and post-translationally modified peptides. *Frontiers in Microbiology*. 2018; 9: 01801. Available from: https://doi.org/10.3389/fmicb.2018.01801.

[75] Qiao W, Qiao Y, Liu F, Zhang Y, Li R, Wu Z, et al. Engineering *Lactococcus lactis* as a multi-stress tolerant biosynthetic chassis by deleting the prophage-related fragment. *Microbial Cell Factories*. 2020; 19(1): 225. Available from: https://doi.org/10.1186/s12934-020-01487-x.

[76] Jiménez JJ, Diep DB, Borrero J, Gútiez L, Arbulu S, Nes IF, et al. Cloning strategies for heterologous expression of the bacteriocin enterocin A by *Lactobacillus sakei* Lb790, *Lb. plantarum* NC8 and *Lb. casei* CECT475. *Microbial Cell Factories*. 2015; 14(1): 166. Available from: https://doi.org/10.1186/s12934-015-0346-x.

[77] Harahap IA, Suliburska J, Karaca AC, Capanoglu E, Esatbeyoglu T. Fermented soy products: A review of bioactives for health from fermentation to functionality. *Comprehensive Reviews in Food Science and Food Safety*. 2025; 24(1): e70080. Available from: https://doi.org/10.1111/1541-4337.70080.

[78] Gontsarik M, Mansour AB, Hong L, Guizar-Sicairos M, Salentinig S. pH-responsive aminolipid nanocarriers for antimicrobial peptide delivery. *Journal of Colloid and Interface Science*. 2021; 603: 398-407. Available from: https://doi.org/10.1016/j.jcis.2021.06.050.

[79] Rajpoot K, Jain SK. Oral delivery of pH-responsive alginate microbeads incorporating folic acid-grafted solid lipid nanoparticles exhibits enhanced targeting effect against colorectal cancer: A dual-targeted approach. *International Journal of Biological Macromolecules*. 2020; 151: 830-844. Available from: https://doi.org/10.1016/j.ijbiomac.2020.02.132.

[80] European Food Safety Authority. EFSA statement on the requirements for whole genome sequence analysis of microorganisms intentionally used in the food chain. *EFSA Journal*. 2021; 19(7): e06506. Available from: https://doi.org/10.2903/j.efsa.2021.6506.

[81] Brodmann T, Endo A, Gueimonde M, Vinderola G, Kneifel W, De Vos WM, et al. Safety of novel microbes for human consumption: practical examples of assessment in the European Union. *Frontiers in Microbiology*. 2017; 8: 01725. Available from: https://doi.org/10.3389/fmicb.2017.01725.

[82] Liao H, Ji Y, Sun Y. High-resolution strain-level microbiome composition analysis from short reads. *Microbiome*. 2023; 11(1): 183. Available from: https://doi.org/10.1186/s40168-023-01615-w.

[83] Zhang M, Qiu X, Zhang H, Yang X, Hong N, Yang Y, et al. *Faecalibacterium prausnitzii* inhibits interleukin-17 to ameliorate colorectal colitis in rats. *PLOS ONE*. 2014; 9(10): e109146. Available from: https://doi.org/10.1371/journal.pone.0109146.

[84] Zhao S, Xiang J, Abedin M, Wang J, Zhang Z, Zhang Z, et al. Characterization and anti-inflammatory effects of *Akkermansia muciniphila*-derived extracellular vesicles. *Microorganisms*. 2025; 13(2): 464. Available from: https://doi.org/10.3390/microorganisms13020464.

[85] Bampidis V, Azimonti G, Bastos ML, Christensen H, Durjava M, Dusemund B, et al. Scientific opinion on the presence of viable cells in a feed additive consisting of lactic acid produced by *Weizmannia coagulans* DSM 32789 (Jungbunzlauer SA). *EFSA Journal*. 2023; 21(4): e07965. Available from: https://doi.org/10.2903/j.efsa.2023.7965.