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The Science of Kefir: Advances in Microbial Ecology, Bioprocess Control, and Functional Health Outcomes

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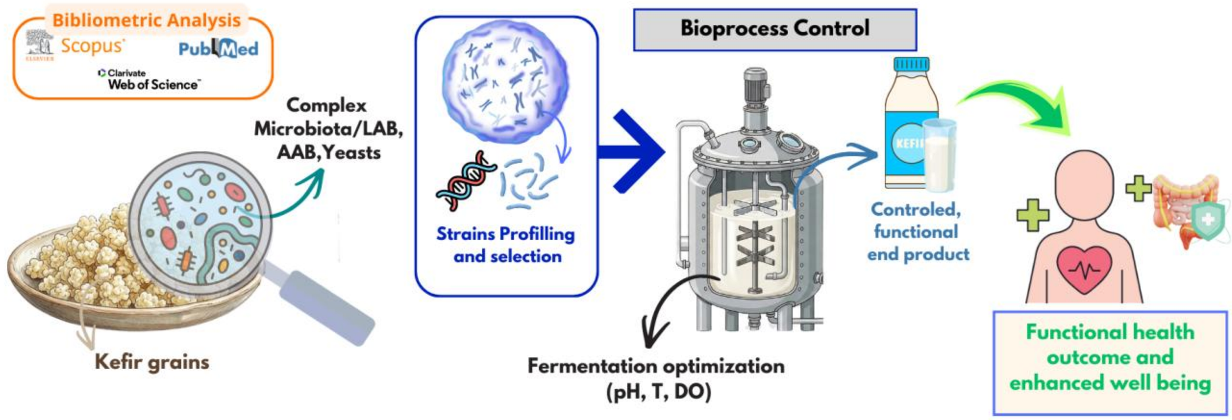
Graphical Abstract

This review analyses current publication trends to outline the development of kefir as a functional food. It examines the complex microbial community and its protective matrix to show how careful strain selection and controlled bioprocesses can improve fermentation and scale up production. Finally, these production methods are linked to human health benefits, highlighting how kefir influences the gut microbiome to support digestion, cognitive function, and overall metabolic balance.



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Abstract: Kefir is a traditional fermented beverage produced by the metabolic cooperation of lactic acid bacteria, acetic acid bacteria and yeasts within a kefiran matrix. This microbial diversity shapes its distinctive sensory and therapeutic properties and has established kefir as a model system in microbial ecology and biotechnology. Guided by the PRISMA framework, this review combines bibliometric analysis with a synthesis of recent work on kefir community dynamics. Metabolic cross-feeding and functional redundancy sustain the stability of this self-organizing ecosystem. The shift from dairy substrates to plant-based matrices is examined in relation to reported health effects, including gut microbiome modulation, immune regulation and antimicrobial activity. Concerns over safety and standardization in spontaneous fermentation have encouraged the development of defined synthetic microbial consortia (SynComs) and controlled bioreactor systems. Emerging tools, including AI-assisted bioprocessing and digital-twin modelling, further position kefir as a model for microbiome-inspired precision biotechnology.

Keywords: *Kefir, Microbial ecology, Bioprocess engineering, Probiotics.*

Introduction

Kefir is a complex, diverse microbial fermented beverage that has entered the market and is also a focus for modern biotechnological and nutritional research. Today, scientific inquiry recognizes kefir as a living model of microbial symbiosis, embodying the integration of diverse microbial ecosystems [1,2].

At the core of kefir's uniqueness are kefir grains, which function as a symbiotic culture of bacteria and yeasts (SCOBY). These grains are not mere microbial suspensions, but highly organized, gelatinous structures consisting of lactic acid bacteria, acetic acid bacteria, and yeasts embedded in an exopolysaccharide matrix known as kefiran. Kefiran not only provides the grains' physical integrity but also contributes to their biofilm-like resilience, supporting microbial coexistence and succession throughout fermentation [2]. This consortium exemplifies microbial diversity and syntrophy, setting kefir apart from other fermented products such as yogurt or kombucha [3].

The scientific and commercial interest in kefir stems from its extraordinary microbial diversity and the health-promoting metabolites it produces, including organic acids, bioactive peptides, and antimicrobial compounds [3]. This diversity has stimulated work showing that kefir consumption benefits gut health, immune modulation, and metabolic balance [3,4]. In parallel, biotechnology has begun to harness kefir's ecological complexity for innovation, ranging from synthetic microbial consortia (SynComs) to precision fermentation platforms. This review therefore aims to synthesize knowledge across traditional microbial ecology, modern bioprocess engineering, and health research, highlighting both foundational science and future opportunities for kefir as a bioengineered functional food [3].

Bibliometric Insights into Kefir Research

Materials and Methods

This review followed a systematic approach guided by the PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) framework, ensuring methodological transparency and minimizing bias. The protocol was structured into four stages to provide a reliable evidence base for researchers, industry stakeholders, and policymakers.

Search strategy

A comprehensive literature search was conducted across three major databases: Scopus, Web of Science, and PubMed. Consistent keyword sets were applied across platforms to maximize retrieval and reduce selection bias (Table 1). The search was restricted to publications between 2015 and 2025, with eligibility criteria focusing on kefir's microbial ecology, biotechnological innovations, nutritional and sensory characteristics, and health-related outcomes. Both experimental and review articles were considered, but non-peer-reviewed sources and non-English publications were excluded. Table 2 summarizes the detailed inclusion and exclusion criteria.

Table 1. Research approach for systematic review process.

Database	Research Approach
Scopus	Title-Abstract-Keywords: ("kefir" OR "kefir grains" OR "fermented milk beverage") AND ("microbial ecology" OR "fermentation dynamics" OR "synthetic microbial consortia" OR "bioprocess engineering") AND ("nutritional composition" OR "sensory properties" OR "health benefits")
Web of Science	Title-Abstract-Keywords: ("kefir" OR "kefir grains" OR "fermented milk beverage") AND ("microbial ecology" OR "fermentation dynamics" OR "synthetic microbial consortia" OR "bioprocess engineering") AND ("nutritional composition" OR "sensory properties" OR "health benefits")
PubMed	Title-Abstract-Keywords: ("kefir" OR "kefir grains") AND ("gut microbiota" OR "probiotics" OR "immune modulation" OR "clinical trial" OR "therapeutic potential")

Selection Criteria

The selection process was conducted in accordance with the PRISMA statement to ensure transparency and reduce selection bias. The search targeted literature on kefir's microbial ecology, biotechnological applications, nutritional and sensory properties, and health-related outcomes. Only publications from 2015 to 2025 were considered, ensuring the review captured the most recent advances. Articles published prior to 2015 were excluded. The search was limited to English, which led to the removal of a large number of records outside this scope. After initial screening, a refined dataset of publications was obtained for further analysis.

Quality Assessment

To maintain scientific rigour, the review included only peer-reviewed original research articles and review papers. Duplicate entries were eliminated through automated and manual screening. The scope was deliberately narrowed to studies directly relevant to kefir fermentation, microbial community dynamics, bioprocess control, product characterization, and therapeutic potential. Publications only tangentially related by keywords (e.g., general dairy microbiology, unrelated probiotics, or nonfermented foods) were excluded to avoid thematic drift. Table 2 summarizes the inclusion and exclusion criteria. Figure 1 illustrates the overall selection process, following PRISMA guidelines.

Table 2. Inclusion and exclusion criteria for the systematic review.

Criteria	Inclusion	Exclusion
Publication period	Between 2015 and 2025	Before 2015
Language	English, French	Languages other than English or French
Document type	Articles, reviews	Reports, book chapters, theses, abstracts

Data Extraction

From the selected records, bibliographic data were extracted, including author names, article titles, abstracts, keywords, publication year, journal source, citation counts, and author affiliations. These metadata supported the identification of research output trends, collaboration networks, keyword co-occurrence, and citation impact. Data extraction was performed consistently across Scopus, Web of Science, and PubMed to ensure comparability.

Bibliometric Analysis

The bibliometric analysis was designed to provide a comprehensive overview of the global research landscape on kefir. Metadata from publications between 2020 and 2025 were analysed to identify emerging themes, collaboration networks, and knowledge clusters. VOS viewer (version 1.6.17) was employed to conduct keyword co-occurrence mapping, co-citation analysis, and bibliographic coupling. Python (version 3.x) generated descriptive visualizations of publication trends and citation metrics with libraries such as matplotlib, pandas, and networkx. This approach enabled the identification of conceptual linkages between kefir's microbial ecology, technological innovation, and health applications, highlighting research hotspots and areas for future exploration.

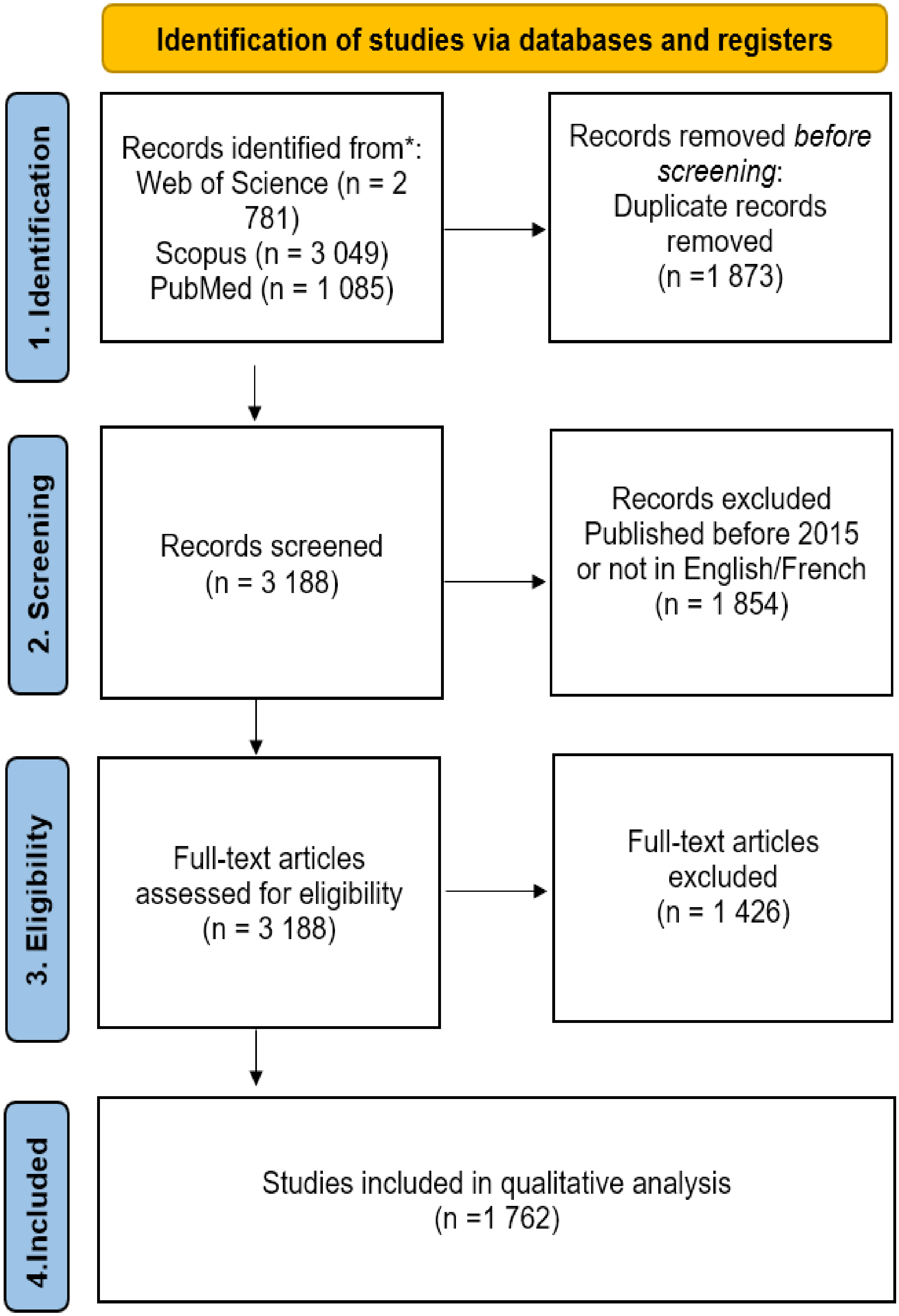

Figure 1. PRISMA 2020: flow diagram for new systematic reviews which included searches of databases and registers.

Bibliometric Results and Discussion

Geographical Distribution of Publications

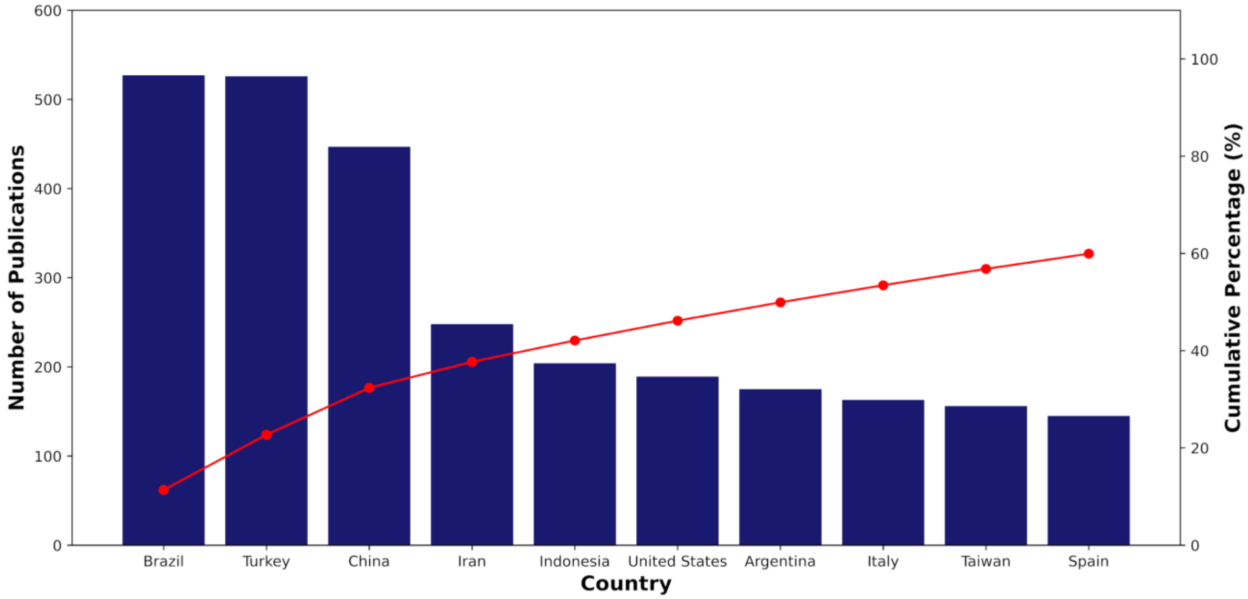

Figure 2. Publication distribution of reviewed papers by country.

As illustrated in Figure 2, kefir research is geographically concentrated. Brazil, Turkey, and China lead global publication output. These countries collectively account for nearly half of all indexed studies, reflecting strong traditions in dairy fermentation and functional food biotechnology. Emerging contributors such as Iran and Indonesia demonstrate growing research capacity. Nations like the United States, Argentina, Italy, Taiwan, and Spain represent secondary hubs emphasizing biotechnological innovation and health-oriented studies. Overall, the top ten countries produce about 60% of kefir-related publications, underscoring both the globalization of kefir research and the persistence of regional research leadership.

Citation Trend Analysis (2020–2025)

As shown in Figure 3, kefir research has experienced fluctuating but generally strong citation activity over the past decade. Citation counts increased notably between 2018 and 2020, peaking in 2019 with over 5,200 citations, reflecting heightened global attention to probiotics and microbiome research during this period. Although a moderate decline is observed after 2021, probably because of publication saturation and the time lag in citation build-up, the overall trend indicates sustained scholarly interest.

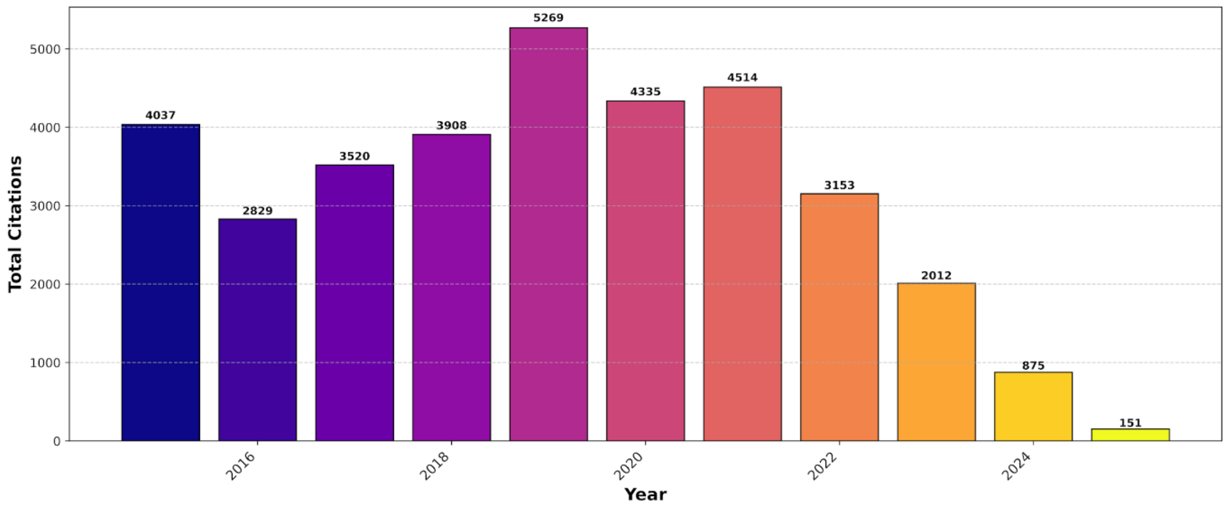

Figure 3. Citation trend for kefir related researches**.**

Keyword Co-occurrence and Thematic Structure of the Field

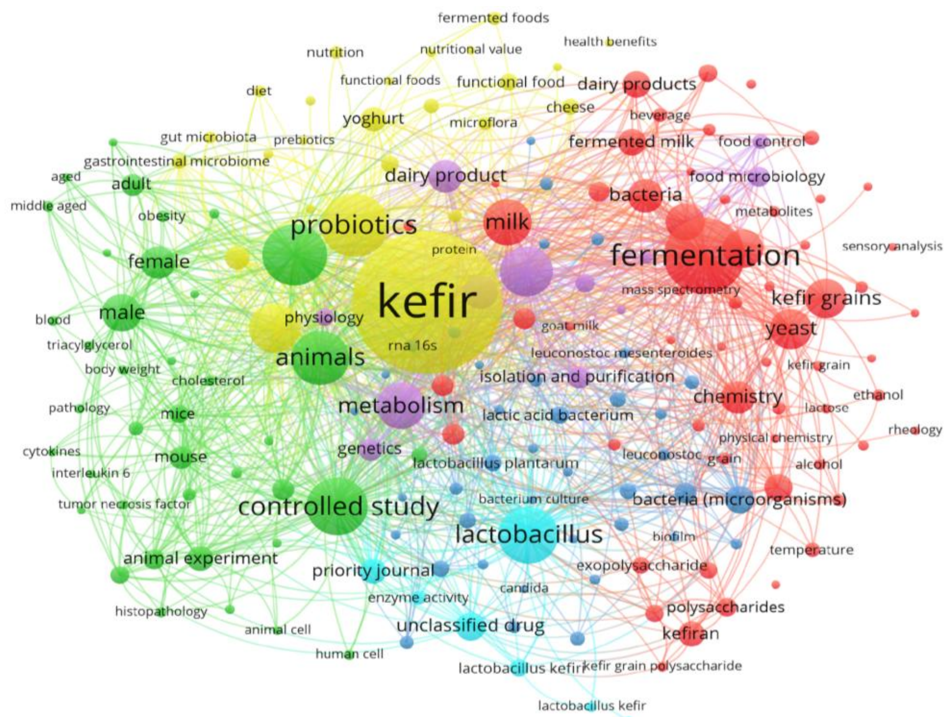

Figure 4. Co-occurrence analysis of author's keyword in VOS viewer.

The co-occurrence network generated through VOS viewer (Figure 4) illustrates the conceptual landscape of kefir research, revealing its interdisciplinary structure spanning fermentation science, microbiology, biotechnology, and health applications. The red cluster, dominated by terms such as fermentation, kefir grains, and yeast, represents the technological and biochemical core of kefir production, emphasizing metabolic interactions and process optimization within the symbiotic community. Closely connected, the blue cluster centres on Lactobacillus, kefiran, and exopolysaccharides, highlighting research focused on structural polysaccharides, biofilm formation, and the physicochemical properties that define kefir’s texture and rheology. The green cluster, encompassing probiotics, metabolism, animals, and controlled study, reflects growing biomedical interest, particularly in kefir’s probiotic potential, immunomodulatory activity, and gut microbiota modulation. Meanwhile, the yellow cluster linking nutrition, functional foods, and health benefits situates kefir within the broader functional food domain, underscoring its integration into nutrition science and public health. Together, these interlinked clusters describe kefir as a model system bridging traditional fermentation and modern biotechnological innovation, connecting microbial ecology, process engineering, and therapeutic functionality within a unified research framework.

Traditional and Modern Kefir: Origins, Types, and Microbial Ecology

Kefir is one of the oldest fermented beverages known in human history, originating in the Caucasus where milk was traditionally fermented in bags made from the hide of goats or cows [3]. Historical accounts describe the passing of kefir grains across generations, showing early ethno-microbiological knowledge of self-sustaining microbial ecosystems. By the nineteenth century, kefir had made its way into Eastern Europe, Russia, and the Middle East, where it became a cultural food as well as a therapeutic agent [2].

Modern kefir exists in two main forms, milk kefir and water kefir, each supported by a complex symbiotic community of bacteria and yeasts (SCOBY) held within an exopolysaccharide matrix. Although both types exhibit comparable ecological organization, they differ markedly in composition, dominant taxa, and metabolic output (Table 3).

Kefir Grains: Structure, Formation, and Microbial Ecology

Kefir’s scientific identity lies in its grains, gelatinous, cauliflower-like aggregates acting as self-propagating natural starters for both milk and water kefir. These grains are complex biofilms comprising lactic acid bacteria (LAB), acetic acid bacteria (AAB), and yeasts enclosed in a polysaccharide–protein matrix mainly composed of kefiran [5,6,7].

Morphology and Ultrastructure

Kefir grains vary from 1 mm to 3 cm, displaying white opaque (milk) or translucent amber (water) appearances. Scanning electron microscopy (SEM) reveals a layered architecture: an outer kefiran-rich shell, a mid-zone of metabolically active LAB, and an inner yeast–AAB core that supports both aerobic and anaerobic microenvironments [8]. This spatial organization ensures functional diversity and structural resilience [8,9].

Genesis and Propagation

Grain biogenesis begins with *Lactobacillus kefiranofaciens* secreting kefiran, forming a fibrillar exopolysaccharide matrix that entraps yeasts and AAB [10]. Repeated fermentation cycles cause aggregation of casein micelles, lipids, and microbial cells, gradually producing a firm three-dimensional network.

In milk kefir, calcium–casein complexes reinforce the grain, whereas in water kefir, dextran and levan-type polysaccharides synthesized by *L. hilgardii* and *Zymomonas mobilis* provide equivalent support [11].

Upon inoculation into fresh substrate, grains expand by **autocatalytic growth**, maintaining compositional balance. Kefiran's vitrification properties confer resistance to drying and freezing, allowing long-term storage without microbial loss [12].

Chemical Composition

On a dry-weight basis, kefir grains contain ~50–60 % carbohydrates, 20–25 % proteins, and 8–10 % lipids, with abundant Ca, Mg, and P minerals [9]. The primary carbohydrate is kefiran, an exopolysaccharide that imparts high viscosity and water-binding capacity to the matrix [7]. Proteins (caseins and microbial surface proteins) are crosslinked through Ca^{2+} bridges, enhancing rigidity; fructan-type exopolysaccharides in water kefir play analogous roles [13].

Microbial Ecology and Symbiosis

Kefir grains function as self-organized ecosystems containing 20 to 50 species. LAB dominate (≈80 %), notably *L. kefiranofaciens*, *L. kefiri*, *Lactococcus lactis*, and *Leuconostoc mesenteroides*. Yeasts (*S. cerevisiae*, *K. marxianus*) and AAB (*A. fabarum*, *A. orientalis*) contribute to ethanol, CO_2, and acetic acid production (Figure 5) [5].

Cross-feeding mechanisms stabilize the consortium: LAB produce lactic acid and peptides used by yeasts, yeasts generate vitamins and ethanol, oxidized by AAB to acetic acid, AAB regulate pH and flavour. These syntrophic networks act as metabolic feedback systems maintaining ecological balance [6,11].

16S rRNA and ITS analyses confirm stable functional guilds across global kefir sources, preserving fermentation dynamics despite compositional shifts [8].

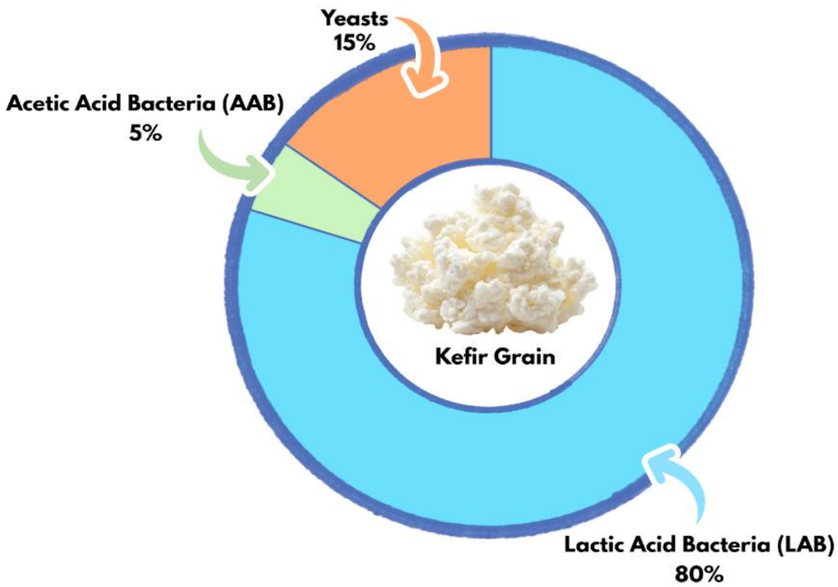

Figure 5. Kefir Grain approximate composition

Comparative Grain Systems

Both milk and water kefir grains display homologous architecture, differing mainly in substrate and dominant taxa [9].

Table 3. Comparative characteristics of milk and water kefir grains

Property	**Milk Kefir Grains**	**Water Kefir Grains**	**References**
Dominant LAB	*L. kefiranofaciens*, *L. kefiri*	*L. hilgardii*, *L. nagelii*	[5,11]
Major Yeasts	*K. marxianus*, *S. kefir*	*S. cerevisiae*, *Candida lambica*	[6,9]
AAB Species	*A. fabarum*, *A. orientalis*	*A. tropicalis*, *Z. mobilis*	[8,11]
Major Exopolysaccharide (EPS)	Kefiran (glucose–galactose)	Dextran/Levan (fructose-based)	[7,13]
Structural Reinforcement	Casein–Ca^{2+} bridges	Sucrose, fruit minerals	[11]
Morphology	White, cauliflower-like	Amber, crystalline	[9,12]
Fermentative metabolism	Lactic–alcoholic–acetic	Alcoholic–acidic–carbonic	[5,11]

Microbial Interactions and Functional Metabolism

Kefir's exceptional metabolic stability arises from its diversified microbial cooperation, behaving as a self-organizing consortium that dynamically balances carbon, nitrogen, and redox fluxes across taxa [6,7]. Within this ecosystem, lactic acid bacteria (LAB), yeasts, and acetic acid bacteria (AAB) coordinate a multi-layered cross-feeding network. LAB dominate early fermentation by hydrolyzing lactose into glucose and galactose, producing lactic acid and peptides that lower the pH and initiate microbial succession. These by-products serve as substrates for yeasts (e.g., *Kluyveromyces marxianus* and *Saccharomyces cerevisiae*), which ferment residual sugars to ethanol and CO_2, while secreting B-vitamins that sustain bacterial growth, while AAB (e.g., *Acetobacter orientalis* and *A. fabarum*) oxidize this yeast-derived ethanol into acetic acid, regenerating NAD^+ and establishing an internal redox equilibrium [5,9].

This cooperative syntropy mimics the ecological interactions of complex environments like soil or gut consortia [14]. Multi-omics and metaproteomic profiling demonstrate that these LAB–yeast–AAB networks form stable biofilms where approximately one-third of the carbon processed by LAB is indirectly respired via yeast and AAB metabolic pathways, highlighting profound energy co-dependence [7,14].

This complementarity extends beyond carbon to a tightly closed nitrogen cycle. Proteolytic LAB, including *Lactobacillus kefiri* and *L. paracasei*, express cell-envelope proteases (CEPs) such as PrtP that hydrolyze caseins into oligopeptides and free amino acids [15,16]. These amino acids stimulate yeast metabolism and drive the biosynthesis of vitamins that reciprocally enhance bacterial proliferation [17]. During late fermentation, yeast autolysis releases amino nitrogen that is reused by LAB, supporting biomass maintenance under nutrient-limited conditions [15].

Furthermore, pH and redox stabilization are intricately linked. While LAB drive initial acidification, heterofermentative species divert pyruvate toward acetate and ethanol, preventing over-acidification. AAB subsequently buffer the medium by oxidizing ethanol to CO_2 and water, sustaining enzymatic activity within an optimal pH range of 4.2–4.5 [16]. Micro-aeration at the grain surface also promotes AAB catalase and peroxidase activity, mitigating oxidative stress and protecting deeper anaerobic LAB niches [17]. Recent evidence even suggests interspecies electron transfer (IET) via redox-active compounds like flavins may occur between yeasts and AAB, enhancing ATP efficiency without excessive acid accumulation [14].

Ultimately, this fine-tuned synergy allows kefir to function as a naturally evolved bioreactor. Beyond primary energy production, co-metabolism generates secondary metabolites including exopolysaccharides (e.g., kefiran), bacteriocins, bioactive peptides, and volatile esters that confer kefir's characteristic sensory and functional complexity [7,18]. When faced with environmental perturbations, acid-tolerant taxa act as stabilizers, granting the system the ecological robustness to persist as a living, self-sustaining network rather than a static fermentation [6,7].

Kefiran and the Biofilm Matrix: Structure–Function Nexus

Building on this metabolic cross-feeding network, the structural integrity of the kefir ecosystem relies entirely on its kefiran matrix. Produced primarily by *Lactobacillus kefiranofaciens*, and to a lesser extent *L. kefiri* and *L. paracasei*, this biopolymer serves as the primary architectural foundation of the grain [4,19,20].

Chemically, kefiran is a branched glucogalactan consisting of nearly equimolar D-glucose and D-galactose residues connected mainly through β-(1→6) and α-(1→2) linkages [21]. Its molecular weight typically ranges from 1.0–3.0 × 10^6 Da, varying with strain, substrate, and fermentation conditions [22]. Advanced FTIR, ^{1}H-NMR, and X-ray diffraction analyses reveal temperature- and pH-dependent conformational transitions, where kefiran chains reorganize from random coils into double-helix domains [20,25]. This semi-crystalline adaptability enables it to act as a natural viscoelastic hydrogel, driving emerging biomedical applications such as wound dressings and controlled drug-delivery matrices [23,24].

At the microbial scale, this matrix regulates the spatial organization required for the consortium's survival. Scanning and confocal microscopy reveal that kefiran forms fibrillar sheaths that anchor cells through electrostatic and hydrophobic interactions [7,26]. By maintaining stable micro-gradients of oxygen, pH, and nutrients, the matrix permits aerobic and anaerobic metabolisms to coexist in spatial harmony. Yeasts adhere to the interior matrix via mannoproteins, while *Acetobacter* species colonize the oxygen-rich outer layers, forming a stratified biofilm analogous to natural ecosystems like lichens or dental plaques [6,27].

The biosynthesis of this polymer is a two-stage enzymatic process: membrane-bound glycosyltransferases polymerize UDP-glucose and UDP-galactose, followed by extracellular secretion through the Wzx/Wzy-dependent pathway [5,28]. The *kef* gene cluster encoding these enzymes shares homology with EPS operons in *Lactobacillus rhamnosus*, though kefiran exhibits higher branching complexity [8]. Crucially, gene expression is stimulated by moderate acid stress (pH 5.0–5.5) and the presence of yeast metabolites such as ethanol and amino nitrogen, demonstrating cross-species signalling control over polymer synthesis [10,29].

Beyond its structural role, kefiran bridges physical bioprocessing and human health. Rheologically, it imparts pseudoplastic and thixotropic properties to fermented beverages, with viscosity correlating linearly with kefiran concentration and chain length [19]. In dairy matrices, it interacts with casein micelles and whey proteins to produce stable emulsions superior to those achieved by xanthan or guar gums [3,30]. Biologically, it exhibits antioxidant, antitumor, and immunomodulatory properties by enhancing macrophage IL-10 expression and suppressing TNF-α [2,31]. Furthermore, kefiran oligosaccharides show targeted prebiotic potential, selectively stimulating *Bifidobacterium adolescentis* and *Lactobacillus plantarum in vitro* [32].

Ultimately, kefiran transforms kefir from a simple liquid fermentation into a highly organized, self-assembling macroscopic structure capable of environmental buffering and profound biological reciprocity.

From Grains to Bioreactors: Controlled and Synthetic Fermentation

Traditional kefir relies on spontaneous grain propagation, but the demands of reproducibility, scalability, and regulatory compliance have catalysed a shift toward controlled fermentation systems and synthetic microbial consortia (SynComs). These engineered approaches emulate the ecological logic of natural kefir while providing predictable functionality and product consistency [33,34].

In artisanal production, microbial composition drifts with environmental factors such as substrate composition, temperature, and storage, leading to sensory and compositional variability. Industrial adaptation therefore begins by decoupling grain maintenance from product fermentation, often through two-stage bioreactors that propagate grains under optimal growth conditions before transferring their metabolites or microbial suspensions into controlled fermenters [35,36].

To overcome the unpredictability of natural grains, several groups have developed defined synthetic consortia reproducing kefir's main ecological and metabolic functions. Minimal communities combining *Lactobacillus kefiranofaciens* (acidification + EPS synthesis), *Kluyveromyces marxianus* (aroma + ethanol), and *Acetobacter orientalis* (redox regulation + acetic acid formation) achieve comparable flavour and texture profiles to traditional kefir with greater batch stability [37-39]. These SynComs mimic kefir's metabolic modularity, wherein each member fulfils a discrete role, acidification, aroma generation, or oxidative buffering, while maintaining cooperative feedback loops [7].

Multi-omics integration has become central to rational SynCom design. Metagenomics and meta transcriptomics reveal the genetic repertoire of natural kefir grains, and metabolomics links pathway activity to flavour compound synthesis. Integrative network modelling identifies keystone interactions such as amino-acid cross-feeding between *Lactobacillus kefiranofaciens* and *Saccharomyces cerevisiae* that are essential for maintaining fermentation stability [40,41]. Through this systems-level approach, researchers have reconstructed kefir-like synthetic consortia capable of predictable acidification kinetics, ethanol yields, and exopolysaccharide production [42]. Table 4 summarizes the transition from traditional culture-based methods to these advanced molecular and cellular profiling techniques.

Table 4. Comparison of traditional and advanced analytical techniques for microbial quality control and community profiling in kefir biomanufacturing

Analytical Approach	Specific Techniques	Key Advantages	Major Limitations	Application in Kefir Biomanufacturing	Reference
Traditional Culture-Based Analysis	• Selective agar plating • Morphological characterization • Biochemical assays	• Highly standardized • Cost-effective • Isolates viable strains for downstream propagation.	• Time-consuming (48–72h) • Underestimates diversity • Fails to detect viable but non-culturable (VBNC) states.	• Baseline enumeration of lactic acid bacteria (LAB) and yeasts • Routine hygiene and purity checks.	[5,9]
Targeted Molecular Quantification	• Quantitative PCR (qPCR) • Reverse Transcription qPCR (RT-qPCR)	• Rapid turnaround (hours) • Highly specific • Capable of quantifying unculturable taxa.	• Requires prior knowledge of target sequences (specific primers) • Limited capability for novel strain discovery.	• Real-time tracking of specific probiotic strains • Rapid detection of spoilage organisms or pathogens.	[8,37]
Next-Generation Sequencing (NGS)	• 16S rRNA / ITS Amplicon Sequencing • Shotgun Metagenomics	• Comprehensive, untargeted profiling of entire consortia • Detects antimicrobial resistance	• High cost, computationally intensive data analysis • Standard DNA-based NGS does not differentiate live vs. dead cells.	• Deep ecological mapping • Validating composition of synthetic microbial consortia (SynComs)	[7,40,41]

		(AMR) genes • Maps metabolic pathways.		• Lot-to-lot consistency validation.	
Advanced Cellular Profiling	• Flow Cytometry (FCM) • Fluorescence In Situ Hybridizatio n (FISH)	• High-throughput, real-time single-cell resolution • Precise quantificati on of live/dead ratios and physiologi cal states.	• High capital equipment cost • Requires specialized technical expertise • Lower taxonomic resolution compared to NGS.	• Rapid viability screening; continuous bioprocess monitoring in smart bioreactors • Stress response analysis.	[41,43]

At the process scale, kefir fermentation has developed into a form of precision microbial manufacturing. Modern bioreactors employ automated pH, temperature, and dissolved-oxygen controls linked to in-line sensors measuring turbidity, redox potential, and metabolite accumulation. Feedback algorithms adjust aeration and feeding rates in real time, ensuring consistent product characteristics. For instance, cascade control strategies that stabilize pH ≈ 4.3 and maintain 0.1–0.2 vvm aeration have been shown to maximize kefiran yield without compromising microbial diversity [43]. Downstream operations such as ultrafiltration, homogenization, and microencapsulation concentrate probiotic biomass and bioactive compounds, extending shelf-life stability [44].

The next frontier will be AI-driven fermentation and digital-twin technologies, coupling real-time sensor data with machine-learning models to predict fermentation trajectories. By integrating omics profiles with environmental variables, these systems can simulate microbial dynamics and enable predictive control and early correction of drift events [45,46]. Such adaptive frameworks align with the emerging paradigm of smart biomanufacturing, in which microbial ecosystems are actively steered toward desired metabolic outputs [47].

Beyond milk substrates, these advances now extend to plant-based kefirs, where controlled inoculation compensates for the absence of lactose buffering and native micronutrients. Engineered consortia optimized for oat, soy, or coconut matrices restore acidification rates and textures comparable to dairy kefir while introducing novel flavour and antioxidant profiles [7,48].

Matrix and Medium Innovations: Milk, Water, and Plant-Based Kefir

Kefir fermentation has diversified far beyond its dairy origins, to become a versatile platform adaptable to sugar-water media and a growing range of plant matrices. Each substrate uniquely shapes microbial dynamics, metabolite output, and functional potential. Innovation in kefir science thus lies not only in microbial control but in the intelligent tailoring of the fermentation matrix to sustain probiotic performance and bioactive production [49,50].

Milk kefir remains the archetype, relying on the dual carbon–nitrogen sources of lactose and casein. As established, this nutrient richness drives the complex proteolytic and cross-feeding networks between *L. kefiranofaciens*, *L. kefiri*, and yeasts [5,7]. The innate buffering capacity of milk stabilizes the pH during fermentation, preserving microbial equilibrium and promoting kefiran biosynthesis. Consequently, dairy kefir maintains a characteristic balance of lactic and acetic pathways, yielding a creamy texture and low ethanol content (<1% v/v) [51].

By contrast, water kefir represents a carbohydrate-driven ecosystem devoid of casein or lactose. Relying on a matrix of sucrose, fruit, and minerals, it is dominated by heterofermentative *Lactobacillus nagelii*, *L. hilgardii*, *Leuconostoc citreum*, and osmophilic yeasts [55]. Here, severe nitrogen limitation is entirely offset by yeast-derived amino acids and vitamins. This mutualism is physically stabilized by dextran-type exopolysaccharides (EPS) synthesized from sucrose, forming a gelatinous network that mimics kefiran functionally but differs in sugar composition [9]. Mineral ions from fruit additions, particularly Ca^{2+}, Mg^{2+}, and Fe^{2+}, enhance microbial adhesion and EPS gelation [8]. Metabolically, this matrix shifts the output markedly toward ethanol (1–2%), CO_2, and organic acids, producing a sparkling, acidic beverage enriched with fruit-derived antioxidants [6].

There is now a trend to plant-based kefirs, where substrates such as oat, soy, coconut, and almond are engineered to mimic milk's nutrient profile and buffering capacity [48]. These matrices present unique carbohydrate bases (e.g., β-glucans, sucrose, raffinose) that require enzymatic adaptation by LAB capable of metabolizing alternative sugars through β-glucosidase or α-galactosidase pathways [51]. To overcome inherent nitrogen deficiencies and support EPS synthesis in lactose-free environments, these systems are frequently optimized through legume extract or protein hydrolysate fortification [12]. When precisely formulated, plant-based matrices achieve acidification kinetics comparable to dairy kefir and generate distinct bioactive profiles, notably elevated γ-aminobutyric acid (GABA) and ferulic acid derivatives that enhance antioxidant and antihypertensive properties [7].

Comparative omics clearly highlight these substrate-specific signatures: milk kefir favours proteolytic LAB; water kefir enriches levan-producing *Lactobacilli*; and plant kefir selects for β-glucosidase-active *Lactiplantibacillus* and *Pediococcus* species [48,54]. These metabolic distinctions drive unique sensory and functional outputs, such as the formation of novel phenolic–microbial conjugates in plant and water kefirs, which expand antioxidant potential beyond that of dairy kefir [13]. Recent innovations even merge these systems into hybrid kefirs (e.g., dairy–oat or coconut–water blends), exploiting complementary microbial niches to create functional beverages enriched with highly diverse prebiotics and postbiotics [48,52,53].

Health Implications and Therapeutic Potential of Kefir Microbiota

The therapeutic potential of kefir extends far beyond its macroscopic nutritional value, operating instead through the dynamic *in vivo* interplay between its live microbial consortium, structural matrix components (e.g., kefiran), and accumulated metabolic by-products [4,56]. Upon ingestion, this complex matrix acts as an active delivery system that collectively modulates host immunity, gut microbiota composition, and metabolic homeostasis. By uniting these nutritional and microbial functionalities, kefir serves as a premier prototype for symbiotic dietary therapy, integrating probiotic, prebiotic, and postbiotic mechanisms into a single biological framework (Figure 6).

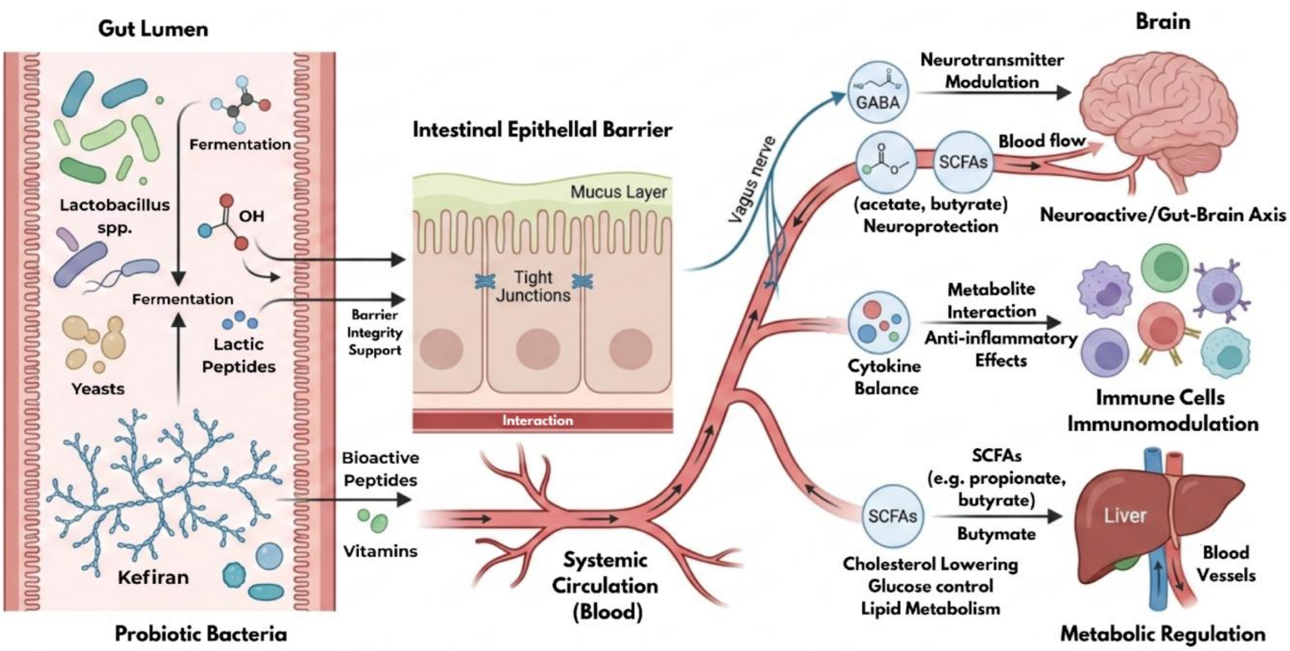

Figure 6. Kefir mechanism of action and health benefits

Gut Microbiota Modulation and Colonization Resistance

The gut microbiome serves as the principal interface through which kefir mediates its systemic benefits. The mixed-microbe consortia of kefir generate a suite of bioactive metabolites including organic acids, bacteriocins, and short-chain fatty acids (SCFAs) that actively restructure intestinal microbial ecology toward enhanced diversity and resilience [35,57]. Regular kefir intake has been linked to increased abundances of beneficial taxa such as *Bifidobacterium* and *Lactobacillus*, while competitively reducing pro-inflammatory groups like *Enterobacteriaceae* and *Clostridium perfringens* [57].

Among the most crucial of these microbial outputs are SCFAs, particularly acetate, propionate, and butyrate, which serve as both vital energy sources for colonocytes and systemic signalling molecules. Locally, these compounds reinforce intestinal barrier integrity by upregulating tight-junction proteins (e.g., occludin, claudin) and reducing epithelial permeability [58,59]. Parallel findings in murine and human trials confirm that this SCFA-mediated pathway extends beyond the gut, modulating broader immune homeostasis and gut-brain signalling [12].

Crucially, the ability of these microbes to reach the lower gastrointestinal tract to exert these benefits is facilitated by the kefiran matrix itself. Kefiran is already established as a potent prebiotic and immunomodulator [60], and its viscoelastic nature also provides a vital biophysical shield *in vivo*. By buffering against severe gastric acidity and bile salts, a property validated through *in vitro* digestion models, the kefiran matrix ensures robust probiotic survival during transit, allowing the active consortia to reach and colonize the intestinal epithelium [7].

Immunomodulatory and Anti-Inflammatory Mechanisms

Kefir’s immunological effects are mediated through a dual action of microbe-associated molecular patterns (MAMPs) and fermentation-derived metabolites. At the cellular interface, microbial surface components such as peptidoglycans, lipoteichoic acids, and yeast-derived β-glucans interact directly with Toll-like receptors (specifically TLR2, TLR4, and TLR9) on intestinal epithelial and immune cells, modulating downstream cytokine signalling cascades [58].

In animal models, this receptor engagement yields a robust systemic immunoregulatory profile. Kefir supplementation actively reduces the expression of pro-inflammatory mediators (IL-6, TNF-α, IFN-γ) while upregulating anti-inflammatory cytokines (IL-10, TGF-β) [5]. Clinically, these shifts translate into protective outcomes in both colitis and allergy models, where kefir administration alleviates epithelial damage and restores mucosal immune balance [6].

At the molecular level, these anti-inflammatory benefits are driven by the kefiran matrix. Building on its previously noted bioactivity, *in vitro* macrophage assays reveal that kefiran specifically suppresses NF-κB nuclear translocation and downregulates inducible nitric oxide synthase (iNOS) expression.

This mechanism effectively restricts the localized production of nitric oxide and reactive oxygen species during inflammatory responses [7].

Furthermore, this immunomodulatory capacity extends systemically into cardiovascular health. The bioactive peptides released during the previously described casein proteolysis act as potent angiotensin-converting enzyme (ACE) inhibitors. By modulating the renin–angiotensin system, these specific microbial metabolites lower vascular inflammation and mitigate hypertension risk, showcasing kefir's multi-organ therapeutic reach [38].

Metabolic and Cardiometabolic Regulation

Kefir's metabolic influence extends comprehensively across glucose and lipid homeostasis, mitigating the oxidative stress central to metabolic syndrome. In both human and animal models, kefir supplementation improves fasting glycaemia and enhances insulin sensitivity. At the hepatic level, it downregulates key gluconeogenic enzymes, specifically phosphoenolpyruvate carboxykinase (PEPCK) and glucose-6-phosphatase (G6Pase), and promoting glycogen synthesis [61-63].

Lipidomic profiling reveals that kefir consumption reduces total circulating cholesterol and triglycerides while elevating HDL-C. This process is driven by the bile salt hydrolase (BSH) activity of resident taxa, such as *Lactobacillus kefiri* and *Leuconostoc* spp., which deconjugate bile acids to decrease intestinal lipid absorption [66]. Furthermore, the short bioactive peptides generated via kefir's inherent proteolysis modulate the PPAR-γ and AMPK signalling pathways, enhancing systemic lipid oxidation and mitochondrial biogenesis [67].

To combat the oxidative damage linked to these metabolic shifts, the synergistic activity of the kefir microbial consortium upregulates the host's endogenous antioxidant defences. By enhancing the expression of enzymes such as superoxide dismutase (SOD) and catalase, kefir significantly reduces malondialdehyde accumulation [64-66]. These intersecting molecular actions make kefir a desirable dietary choice for managing metabolic syndrome and mild hyperlipidemia.

Neuroactive and Psychobiotic Properties

Emerging research puts kefir within the growing field of psychobiotics, microbial interventions targeting mental health via the gut–brain axis. Specifically, kefir-derived strains, notably *Lactobacillus kefiranofaciens* and *Lactiplantibacillus plantarum*, synthesize high levels of the previously noted γ-aminobutyric acid (GABA) alongside other neuroactive compounds that modulate the enteric nervous system and central neurotransmission [68].

In murine models, this microbial-driven neuromodulation significantly reduces circulating corticosterone levels, normalizes hippocampal serotonin, and alleviates anxiety-like behaviours through vagal nerve–dependent pathways [59]. This demonstrates that kefir's bioactive influence extends beyond the gut, sending signals to the central nervous system.

Antimicrobial and Anticancer Activities

Kefir also exhibits potent, direct antimicrobial effects. These result from the synergistic action of synthesized bacteriocins (such as kefiricin), organic acids, hydrogen peroxide, and carbon dioxide. Together, these metabolites actively disrupt and inhibit major foodborne pathogens, including *Listeria monocytogenes*, *Escherichia coli*, and *Salmonella typhimurium* [69].

Recent clinical and *in vitro* reports highlight kefir's potential in targeted onco-prevention. Building on the established antitumor capacity of the kefiran matrix, whole fermented extracts have been shown to induce apoptosis in colon and breast cancer cell lines specifically via targeted caspase activation and reactive oxygen species (ROS) modulation [7].

These specific apoptotic mechanisms align with kefir's broader anti-inflammatory and antioxidant profile [67,69].

Safety Considerations and Regulatory Challenges

Spontaneous fermentation inherently carries safety and commercial standardization challenges [70]. Artisanal kefir grains, maintained in open or semi-controlled environments, are susceptible to fluctuations in community dynamics. This variability can lead to the accumulation of biogenic amines, such as histamine and tyramine, or potential contamination by opportunistic pathogens [71,72].

It is difficult to navigate the regulatory landscape for live biotherapeutics and complex probiotic foods. Although many dominant lactic acid bacteria in kefir are Generally Recognized as Safe (GRAS) by the US Food and Drug Administration (FDA) or possess Qualified Presumption of Safety (QPS) status in Europe, regulatory bodies struggle to classify undefined, mixed-microbe consortia [72]. The intrinsic heterogeneity of kefir grains poses severe challenges for reproducibility and safety validation.

Stringent quality control for kefir requires genomic screening for antimicrobial resistance (AMR) genes, the establishment of strain-specific reference cultures, and harmonized definitions of "traditional," "industrial," and "engineered" kefir to enable credible comparison across regulatory jurisdictions [70]. The development of defined synthetic microbial consortia (SynComs) not only provides process stability but also offers a clearer regulatory pathway by ensuring the reproducibility, safety, and traceability demanded by modern food safety authorities [33,37,38].

Conclusion and Future Questions

Kefir brings together microbial ecology, biochemistry, and biotechnology; it is a living system where traditional fermentation meets modern analytical science. Once 'an ethnic food', it now enters the market as a familiar choice, and also serves as a premier model for studying microbial cooperation, sustainability, and bifunctionality. Its complex community of bacteria and yeasts is a self-regulating ecosystem, with the kefiran matrix providing the structural and metabolic stability essential for both product quality and therapeutic benefits.

Emerging multi-omics and systems-biology approaches are deepening our understanding of kefir's metabolic networks, paving the way for rationally designed microbial consortia with enhanced functionality and resilience. Advances in synthetic biology, Artificial Intelligence, and precision fermentation are transforming kefir production into an intelligent, adaptive bioprocess with profound applications across health, nutrition, and sustainable food systems.

Kefir exemplifies the next generation of functional foods, one that integrates nutrition, ecological balance, and biotechnological innovation. Continued research and standardization will be vital to securing its full potential, not only as a model of microbial synergy but as a definitive blueprint for future bioengineered, health-promoting foods.

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