Recent Advances in Dairy and Food Bio Preservation Emerging Biological Strategies and Technological Integration

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***Abstract*—Food spoilage and pathogenic contamination are persistent challenges for the global food industry, with serious economic and public health consequences. This problem is especially severe in developing nations like India, where cold chain infrastructure remains unreliable. Conventional preservation methods — including synthetic chemical additives and thermal treatments — face growing limitations due to rising consumer demand for clean-label products and the destruction of heat-sensitive bioactive compounds during processing. Bio-preservation, defined as the use of naturally occurring microorganisms and their metabolic products to extend food shelf life and improve microbial safety, offers a scientifically sound and consumer-acceptable alternative. This review examines four major classes of bio-preservative agents: lactic acid bacteria (LAB), bacteriocins, bacteriophages, and endolysins. Their antimicrobial mechanisms, current food applications, key advantages, and practical limitations are discussed in detail. The integration of these biological agents with non-thermal technologies — high pressure processing, ultrasound, and cold plasma — is assessed through the hurdle technology framework. Future directions examined include nano-encapsulation of antimicrobial compounds, metabolically engineered LAB strains, CRISPR-based pathogen targeting, and intelligent active packaging — all with specific relevance to the Indian dairy and food processing sectors. The paper concludes that bio-preservation holds substantial, largely untapped potential in India, and that indigenous LAB strains derived from traditional fermented foods represent a highly promising but underexplored research frontier**

***Index Terms*—Bio-preservation; Lactic acid bacteria; Bacteriocins; Bacteriophages; Endolysins; Hurdle technology; Dairy preservation; Food safety; non-thermal processing; Nisin; Indian dairy; Active packaging**

I. INTRODUCTION

Food preservation is one of the most enduring challenges in human civilisation. Fermentation is among its earliest practical solutions. In Indian households, the daily preparation of dahi from fresh milk is a naturally occurring bio-preservation process. Lactic acid bacteria convert lactose into lactic acid, creating an environment chemically hostile to spoilage organisms, all without synthetic intervention. It is through modern food science that the microbiology underlying such traditional practices becomes fully understood, and potentially harnessed in more deliberate, systematic ways.

Food spoilage causes the loss of approximately one-third of all food produced globally, with developing nations bearing a disproportionate share of this burden (FAO/WHO, 2019). In India specifically, the dairy sector reports post-harvest losses of paneer, khoa, and fresh milk running into thousands of crores of rupees annually. Microbial spoilage, worsened by an inconsistent cold chain — particularly at the last mile of distribution — is the primary cause (ICAR-NDRI, 2022). Paneer, one of the most widely consumed fresh cheeses in India, has a shelf life of only 5–7 days under refrigeration and just 1–2 days at ambient temperatures. Khoa and mawa-based confectionery are equally vulnerable, with significant spoilage occurring between production and retail sale (Singh & Patil, 2023).

Conventional preservation methods each face meaningful constraints. Refrigeration is energy-intensive and inaccessible to small producers across rural India. Heat treatments such as pasteurisation and ultra-high temperature (UHT) processing are effective against microbial loads but alter sensory properties and destroy heat-sensitive nutrients like vitamins B and C. Consumer surveys across multiple markets consistently show declining acceptance of synthetic preservatives, with a growing preference for minimally processed, clean-label foods, a trend accelerating in urban India (Bhattacharyya et al., 2025).

Bio-preservation addresses these limitations by using naturally occurring microorganisms or their metabolic products as preservative agents. The approach is grounded in ecological reality: microbial communities are never static. Beneficial organisms and spoilage or pathogenic ones compete constantly for nutrients, space, and ecological dominance. Bio-preservation deliberately channels this competition in favour of food safety. This review examines four principal classes of bio-preservative agents — lactic acid bacteria, bacteriocins, bacteriophages, and endolysins — alongside their integration with non-thermal processing technologies. The discussion is situated specifically within the Indian food industry context, where the opportunity for bio-preservation is both scientifically and economically compelling.

II. LITERATURE REVIEW METHODOLOGY

Relevant literature from 2015 to 2025, with primary emphasis on publications from 2020 onwards, was identified through systematic searches of Google Scholar, ScienceDirect, PubMed, and Web of Science. Search terms included: "bio-preservation food", "lactic acid bacteria dairy", "bacteriocins food safety", "bacteriophages food application", "endolysins food", "hurdle technology non-thermal", "paneer shelf life", "Indian dairy preservation", and "FSSAI food safety". Additional references were identified through citation tracking of high-impact review articles. Grey literature from FAO/WHO and ICAR was consulted for India-specific data.

Studies were selected based on their relevance to the mechanisms, applications, or limitations of biological preservation agents in food systems. In vitro microbiology studies without direct food application were excluded from the primary analysis but cited where mechanistic context was required.

III. BIOLOGICAL BASIS OF FOOD BIO-PRESERVATION

The scientific rationale for bio-preservation is grounded in competitive microbial ecology. In any food matrix, beneficial microorganisms and spoilage or pathogenic organisms compete for nutritional resources and ecological space. Bio-preservation harnesses this antagonism by deliberately selecting, concentrating, and deploying the most effective inhibitory organisms or their derived products to shift the competitive balance towards food safety.

Fermentation provides the clearest illustration. When LAB convert lactose to lactic acid during dahi production, the resulting pH drop creates conditions under which most common foodborne pathogens — including Salmonella enterica, Listeria monocytogenes, and Staphylococcus aureus — cannot survive or replicate. The food matrix becomes microbiologically hostile, not through an external additive, but through the metabolic output of the bacteria themselves. Indian households have practised this for centuries (Tamang et al., 2020).

Researchers classify bio-preservation strategies into three broad categories. Culture-based methods employ live microbial cultures. Metabolite-based methods apply extracted antimicrobial compounds, such as bacteriocins, directly to food. Enzyme-based methods use purified lytic enzymes such as endolysins. Bacteriophages form a fourth and increasingly important category, deploying viral biology to destroy bacteria with a specificity unmatched by other agents (Devlieghere et al., 2004; Rameez et al., 2024). Each category carries distinct mechanisms, application profiles, and limitations; understanding these distinctions is essential before designing any multi-agent preservation system.

IV. LACTIC ACID BACTERIA (LAB)

Lactic acid bacteria are the cornerstone of food bio-preservation. Their safety record, accumulated through millennia of human consumption across fermented foods globally, is formally recognised through the Generally Recognised as Safe (GRAS) designation by the US

Food and Drug Administration and the Qualified Presumption of Safety (QPS) status by the European Food Safety Authority. These designations reflect an extensive empirical and mechanistic evidence base, not merely regulatory convention.

LAB exert antimicrobial activity through several parallel mechanisms. The primary one is fermentative production of lactic and acetic acids, which reduce pH to levels incompatible with pathogen growth. This acid production is augmented by hydrogen peroxide — a potent oxidising agent — and diacetyl, which disrupts microbial membrane integrity. Beyond chemical inhibition, LAB actively compete for nutritional substrates and colonisation sites, reducing the ecological niche available to pathogens. Many LAB strains also produce bacteriocins — ribosomally synthesised antimicrobial peptides — discussed in Section 5 (Putri et al., 2024; Marcelli et al., 2024).

In the Indian dairy context, LAB strains derived from traditional fermented foods offer particular promise. Tamang et al. (2020) documented extensive diversity of LAB strains from traditional Himalayan fermented products including kinema, gundruk, and regional dahi varieties, many of which showed strong antimicrobial activity against common foodborne pathogens. These domestically adapted strains are likely to perform more reliably in Indian food matrices than commercial starter cultures developed for temperate-climate systems. This represents a commercially and scientifically underexplored area of high value.

In meat preservation, LAB strains have shown measurable efficacy against Pseudomonas species — the principal agents of spoilage in refrigerated meats. For India's rapidly expanding packaged meat sector, this application has direct economic relevance (Marcelli et al., 2024). It must be acknowledged, however, that LAB-based bio-preservation is not without limitations. The speed of inhibition is slower compared to chemical preservatives, and efficacy is strongly strain-dependent, requiring careful selection for each specific food matrix and target pathogen.

V. BACTERIOCINS

Bacteriocins are ribosomally synthesised antimicrobial peptides produced by bacteria — predominantly LAB — as part of their competitive inhibition repertoire. They differ fundamentally from conventional antibiotics: they are not products of complex secondary metabolic pathways, and their antimicrobial activity targets the cell membrane directly rather than inhibiting intracellular metabolic processes. This distinction has important implications for resistance development and regulatory classification.

The primary mechanism involves binding of the bacteriocin peptide to specific receptors on the target cell's outer membrane, followed by insertion into the lipid bilayer and formation of transmembrane pores. This disrupts the electrochemical gradient, causes uncontrolled ion flux and leakage of intracellular contents, and leads to cell death. Nisin — the most commercially established bacteriocin — additionally binds to Lipid II, a critical intermediate in bacterial cell wall biosynthesis. This dual mode of action is a key reason why nisin remains highly effective

even after decades of use, with a notably low propensity for resistance development (Cleveland et al., 2001; Mokoena et al., 2023).

Nisin is currently approved in over 50 countries, including India, where it is used in processed dairy products, certain canned foods, and beverages. Its heat stability makes it particularly suitable for pasteurised dairy products. Despite its effectiveness, bacteriocins face real stability limitations in complex food matrices: they may be degraded by endogenous food-matrix proteases, inactivated by binding to fat or protein components, or reduced in efficacy by pH variation during processing and storage. Pediocin and enterocin are the next generation of LAB-derived bacteriocins under active investigation. Recent controlled trials have demonstrated their efficacy against L. monocytogenes and Salmonella in dairy and meat matrices without detectable sensory impact at effective concentrations (Bhattacharyya et al., 2025; Pujato et al., 2024).

From a consumer-acceptance perspective, bacteriocins carry a meaningful advantage: being proteins, they are fully degraded by gastrointestinal proteases and do not accumulate in the body or in the food chain. This biodegradability distinguishes them sharply from many synthetic preservatives. The regulatory status of bacteriocins beyond nisin remains a commercial barrier — pediocin and enterocin lack equivalent global approval, and the approval pathway through FSSAI is currently lengthy. These challenges are real but not insurmountable, and bacteriocins remain one of the most scientifically mature and commercially viable categories of bio-preservative agents available today.

VI. BACTERIOPHAGES

Bacteriophages are viruses that infect and lyse bacteria. Their specificity is determined by the complementarity between phage tail fibre proteins and receptor molecules on the bacterial cell surface. Each phage strain targets a narrow range of bacterial hosts — often a single species or even a single strain — and this narrow host range is simultaneously the most important advantage and the primary limitation of phage-based bio-preservation.

Phage action follows a defined sequence: specific adsorption to the host cell surface, injection of phage nucleic acid, replication using host cellular machinery, assembly of progeny phage particles, and host cell lysis mediated by phage-encoded endolysins. In lytic phage infections, this cycle completes rapidly — often within 20–30 minutes under optimal conditions — releasing multiple phage progeny capable of infecting additional host cells. In food systems, this amplification effect means that relatively small initial phage doses can achieve major reductions in target pathogen loads (Rameez et al., 2024).

Published studies confirm effective suppression of Salmonella enterica, Escherichia coli O157:H7, and Listeria monocytogenes in ready-to-eat meats, fresh produce, and dairy products using phage preparations. The US FDA has approved several commercial phage products, including ListShield (Intralytix) for Listeria control in ready-to-eat foods. EFSA has similarly authorised phage preparations for specific applications in Europe. In India, however, FSSAI has

not yet established a clear regulatory framework for phage use in food — a major barrier to near-term commercial deployment.

The narrow host range limitation is partially addressed through phage cocktail formulations, which combine multiple phage strains targeting different serotypes of the same pathogen. This broadens coverage without sacrificing specificity and reduces the risk of phage-resistant mutants emerging. Phage specificity remains a double-edged characteristic: it prevents disruption of beneficial microbiota and has no sensory impact on food, but it requires accurate identification of the contaminating pathogen strain — a diagnostic capability not universally available in smaller Indian food production operations. Additionally, phages are inactivated by the high temperatures used in cooking and pasteurisation, limiting their application to post-processing or surface-treatment stages.

VII. ENDOLYSINS

Endolysins are phage-encoded enzymes produced at the terminal stage of the phage replication cycle. Their role is to lyse the bacterial host cell from within, releasing progeny phage into the environment. Their catalytic activity targets peptidoglycan — the principal structural polymer of the bacterial cell wall — through cleavage of specific glycosidic or peptide bonds. When applied externally to food products or food-contact surfaces, endolysins diffuse to the peptidoglycan layer and achieve rapid, targeted lysis of susceptible bacterial cells.

A particularly important property of endolysins is their resistance profile. Peptidoglycan is essential for bacterial structural integrity, and mutations that substantially alter its composition are generally lethal to bacteria. This constraint means that, unlike antibiotics — which target metabolic enzymes or nucleic acid synthesis pathways subject to frequent mutational resistance — endolysins do not readily select for resistant variants. The rate of resistance emergence for endolysins is substantially lower than for conventional antibiotics, which is a notable advantage in the context of growing global antimicrobial resistance (Rameez et al., 2024; Settanni & Corsetti, 2008).

From a processing perspective, endolysins offer a speed advantage: target cell lysis occurs within minutes under appropriate conditions. For food manufacturing environments where rapid surface decontamination is important — processing equipment, conveyor surfaces, packaging stations — this characteristic is directly valuable. Application formats are flexible: spray treatments, incorporation into wash solutions, coating onto packaging materials, and rinse solutions. For Indian small and medium-scale dairy operations, where L. monocytogenes and S. aureus contamination risks are elevated relative to large-scale controlled environments, endolysins could provide a practical, rapid-action intervention that does not require major capital investment.

Production cost remains the most significant barrier to wider endolysin adoption. As recombinant proteins, they require expression through microbial fermentation systems and multi-step downstream purification — a process that, at current scale, generates prohibitive unit costs for many food manufacturers. Research into endolysin production optimisation is accelerating,

however, and unit costs are expected to decline as expression systems improve and production scale increases. Commercial availability at accessible price points is anticipated within the next five to ten years (Rameez et al., 2024).

Table 1: Comparative Overview of Bio-preservative Agents — Mechanisms, Applications, and Advantages

Agent	Mechanism of Action	Primary Applications	Key Advantages
Lactic Acid Bacteria (LAB)	Lactic/acetic acid production; pH reduction; competitive exclusion; bacteriocin secretion	Dahi, paneer, fermented beverages, kinema, meat products	GRAS/QPS status; centuries of safe use; low production cost
Nisin (Bacteriocin)	Lipid II binding; membrane pore formation; cell-wall synthesis inhibition	Processed cheese, canned vegetables, flavoured milk, khoa-based sweets	FDA/EFSA approved; heat-stable; biodegradable; no sensory impact
Pediocin & Enterocin	Membrane disruption; inhibition of Listeria, Salmonella, S. aureus	Meat products, dairy, ready-to-eat processed foods	Effective at low concentrations; no flavour change detected
Bacteriophages	Specific viral infection; bacterial DNA degradation; host cell lysis	Ready-to-eat meats, poultry, fresh produce, processing surfaces	Ultra-specific; self-limiting; no sensory effect on food
Endolysins	Enzymatic cleavage of peptidoglycan; rapid bacterial cell-wall lysis	Dairy surface decontamination, packaging coatings, rinse solutions	Rapid action (within minutes); very low resistance potential; targeted

Figure 1: Mechanisms of Action of Bio-preservative Agents

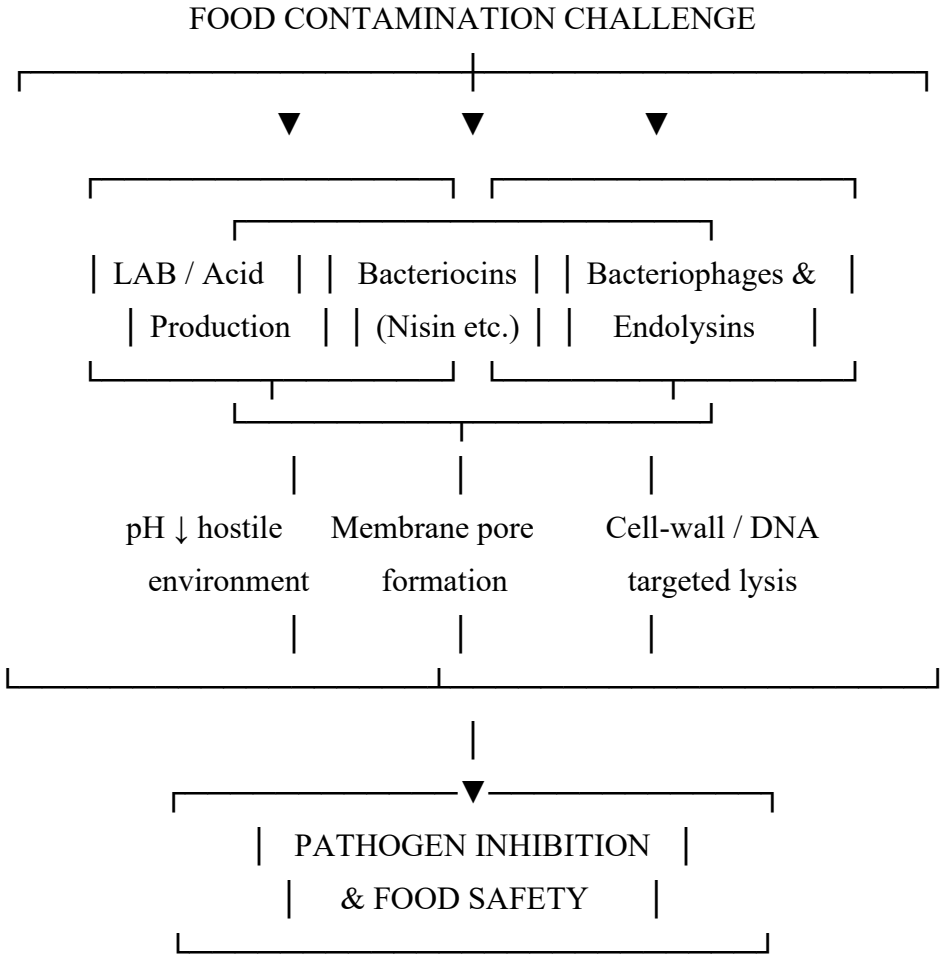

Figure 1: Schematic representation of the primary mechanisms of action of bio-preservative agents in food systems. Each agent operates through a distinct biological pathway, enabling rational multi-agent formulation.

VIII. CHALLENGES AND LIMITATIONS

A rigorous review of bio-preservation must go beyond cataloguing advantages. Realistic assessment requires explicit consideration of the constraints that limit wider adoption. These challenges span technical, regulatory, economic, and social dimensions — and any practical pathway to commercial scale-up must address them directly.

8.1 High Production Cost

LAB starter cultures are relatively inexpensive and already manufactured at large scale for the global dairy industry. The production of bacteriocins, endolysins, and phage preparations, however, involves considerably more complex and costly processes. Bacteriocin extraction from fermentation broth requires precipitation, chromatographic purification, and quality testing — all of which substantially increase unit cost. Endolysins, as recombinant proteins, require expression

system development, fermentation, and multi-step purification to achieve food-grade purity. For small and medium food enterprises — which constitute the overwhelming majority of India's food processing sector — these current costs may be prohibitive without government subsidy support, cooperative production models, or significant economies of scale.

8.2 Regulatory Approval Barriers

Regulatory frameworks for novel bio-preservative agents vary widely across jurisdictions and, in many cases, are still evolving. Nisin holds approval in over 50 countries, but pediocin and enterocin lack equivalent global status. In India, FSSAI's approach to novel food ingredients requires extensive safety dossiers and multi-stage review processes that can span several years. Bacteriophage preparations — approved in the United States and parts of Europe — have no clear regulatory pathway in India at present. Genetically engineered LAB strains face additional barriers under India's biosafety framework, which historically treats GMO food products with considerable caution. These regulatory gaps delay the deployment of scientifically validated technologies in Indian markets.

8.3 Stability in Complex Food Matrices

The efficacy of bio-preservative agents is frequently reduced by the chemical complexity of real food systems. Bacteriocins are susceptible to degradation by endogenous food proteases — particularly in protein-rich dairy matrices — and can be inactivated through non-specific binding to lipid or protein components. This reduces the concentration of active agent available at the site of action. Bacteriophages are sensitive to high temperatures and may denature during heat-treatment steps. The stability of both bacteriocins and phages across the pH range encountered in commercial food processing — from near-neutral fresh milk to acidic yoghurt — varies significantly by product and strain. Nano-encapsulation offers a promising solution to these stability challenges, but its food-grade application requires further development before commercial deployment (Bhattacharyya et al., 2025).

8.4 Narrow Spectrum and Phage Specificity

The high specificity that makes bacteriophages attractive for targeted applications also limits their usefulness in situations of polymicrobial contamination or when the identity of the contaminating strain is unknown. A phage preparation optimised for one Listeria monocytogenes serotype may show no activity against a different serotype on the same production line. Nisin and most individual bacteriocins are effective primarily against Gram-positive bacteria and show little efficacy against Gram-negative pathogens such as Salmonella and E. coli — organisms of major food safety significance globally. Phage cocktail formulations partially address this by combining multiple strains, but the cocktail must be reformulated as pathogen populations evolve, adding operational and regulatory complexity.

8.5 Consumer Acceptance and Communication

Consumer acceptance of bio-preservation technologies is not uniform and is shaped by cultural context, media framing, and prior exposure. LAB-based fermentation is generally well accepted globally because of strong familiarity with fermented foods. The concept of viruses — even non-pathogenic, food-specific bacteriophages — being applied to food may generate concern among consumers unfamiliar with their safety profile. Similarly, genetically engineered LAB strains face resistance in markets with established concerns about biotechnology in food, including India. Transparent, scientifically accurate, and culturally sensitive communication strategies will be essential to building consumer confidence in newer bio-preservative technologies.

8.6 Industrial Scaling Challenges

Many bio-preservative formulations that perform well under controlled laboratory conditions face significant challenges when moving to industrial food production, including variable raw material quality, fluctuating processing temperatures, high throughput volumes, and the need for consistent dosing. Phage cocktails must be stored under controlled conditions to maintain viability. Endolysins require cold storage and careful handling. Standardising production protocols, quality control frameworks, and application methodologies across diverse small and medium-scale Indian food processing units is a practical challenge that most laboratory-scale research publications do not adequately address.

IX. INTEGRATION WITH EMERGING NON-THERMAL TECHNOLOGIES

The limitations of individual bio-preservative agents provide strong justification for combining them with non-thermal processing technologies through the hurdle technology framework. The hurdle concept, first systematically articulated by Leistner (2000), holds that multiple simultaneous mild stresses — each operating through a distinct mechanism — achieve synergistic microbial inactivation that no single intervention could accomplish independently. The key benefit: hurdle technology achieves this enhanced efficacy at lower individual doses of each component, which translates directly into better retention of sensory and nutritional properties and, in some cases, lower overall processing costs.

9.1 High Pressure Processing (HPP)

HPP exposes food to isostatic hydrostatic pressures of 100–600 MPa for short durations, causing irreversible deformation and rupture of microbial cell membranes while preserving covalent bonds — and thus flavour compounds, vitamins, and colour profiles. Cells that survive HPP treatment are structurally compromised and significantly more susceptible to chemical stress from bacteriocins or organic acids. Studies have demonstrated that HPP combined with nisin produces substantially greater log-reductions in Listeria counts than either treatment applied alone, at pressure levels that individually would be insufficient for regulatory-compliant pathogen control (Verraes et al., 2013). HPP is already commercially operational in India for fruit juices and certain packaged foods, providing existing infrastructure on which bio-preservation integration can build.

9.2 Ultrasound Treatment

High-frequency ultrasound generates acoustic cavitation — the rapid formation and violent collapse of microbubbles — producing intense localised mechanical stress and transient temperature spikes. These effects damage microbial cell membranes without raising bulk product temperature, making ultrasound particularly suitable for heat-sensitive foods like fresh paneer, raw milk, and fruit-based beverages. When combined with LAB cultures or bacteriocins, cavitation-induced membrane damage increases permeability to antimicrobial compounds, amplifying their inhibitory effect on surviving cells. Ultrasound also improves homogeneity and reduces microbial load at product surfaces — relevant for whole-piece dairy products like fresh paneer. Limited penetration into dense solid food matrices and challenges in achieving uniform acoustic field distribution at commercial scales are areas that still require engineering development.

9.3 Cold Plasma

Cold plasma is generated by applying energy to gases at near-ambient temperatures, creating a partially ionised medium rich in reactive oxygen and nitrogen species (RONS). These RONS attack both bacterial DNA and lipid membranes simultaneously. Unlike thermal processing, cold plasma does not significantly alter the physical structure of solid foods. When applied with bacteriocins, the oxidative membrane damage induced by RONS reduces the concentration of bacteriocin required for effective cell killing — a synergy with both efficacy and cost benefits. Cold plasma research in food applications is at a relatively early stage compared to HPP or ultrasound, but pilot-scale trials globally have produced encouraging results. For Indian applications, cold plasma is particularly relevant for surface decontamination of spices, fresh produce, and packaging materials (Pujato et al., 2024).

Table 2: Emerging Non-Thermal Technologies for Integration with Bio-preservative Agents

Technology	Mechanism	Advantage	Limitation	Indian Suitability
High Pressure Processing (HPP)	Hydrostatic pressure (100–600 MPa) ruptures microbial membranes	Non-thermal; retains flavour/nutrients; synergises with bacteriocins	High capital cost; batch processing only; limited SME access	Growing — commercial in juice sector
Ultrasound Treatment	Cavitation creates mechanical cell-membrane damage	No heat applied; retains heat-sensitive nutrients; fast action	Limited penetration in dense foods; scale-up challenges	Promising for paneer, raw milk, fruit pulp
Cold Plasma	Reactive	Broad-spectrum	High equipment	Early-stage;

	oxygen/nitrogen species attack bacterial DNA and membranes	antimicrobial; no thermal degradation of nutrients	cost; still at pilot scale in food sector	high future potential for spices, packaging
Pulsed Electric Fields (PEF)	Short high-voltage pulses create irreversible membrane pores	Non-thermal; rapid; liquid food compatible	Less effective on solid foods; equipment investment required	Relevant for milk, juice, liquid dairy

Figure 2: Hurdle Technology Framework for Food Bio-preservation

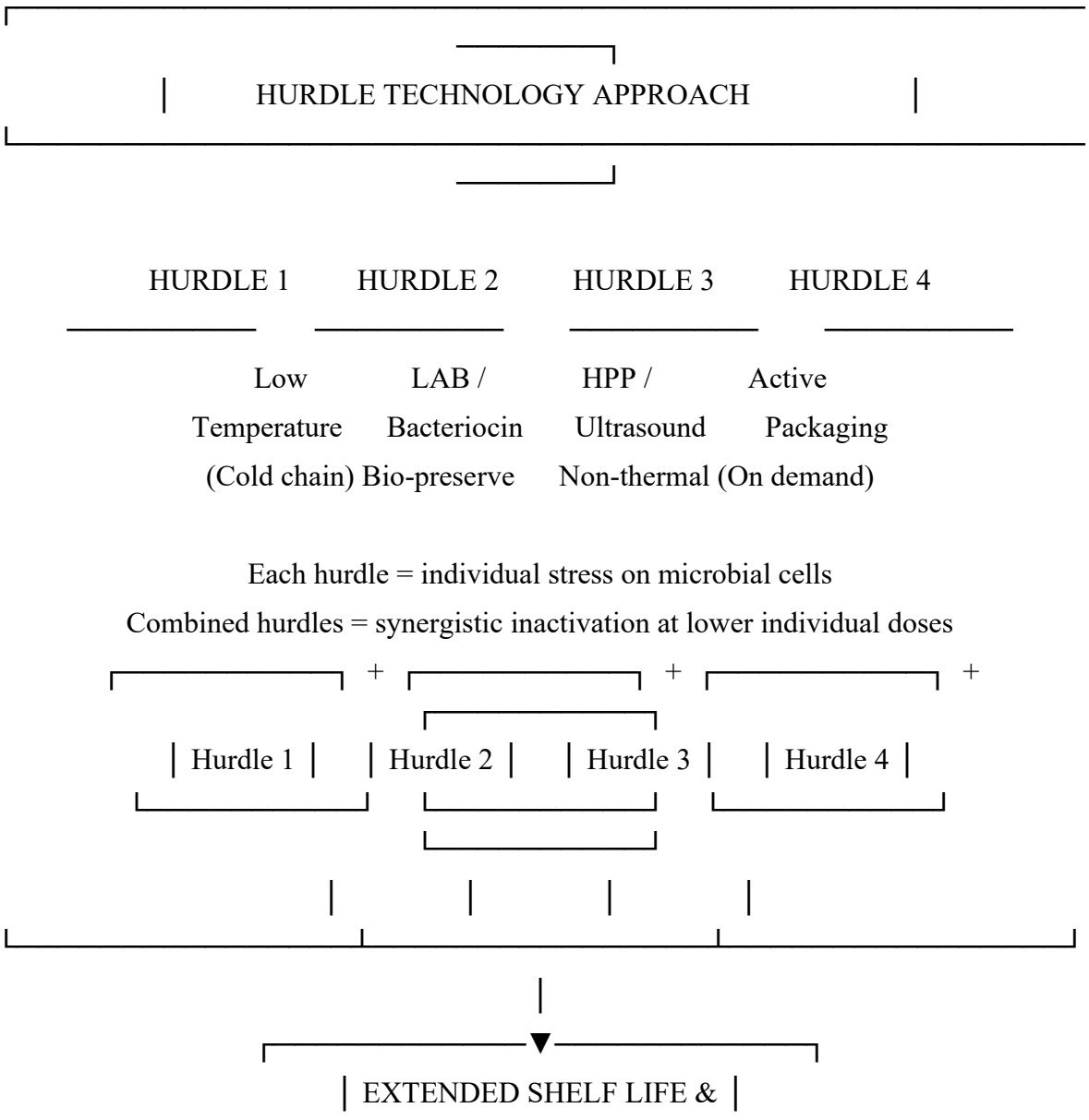

Figure 2: Hurdle technology framework for multi-barrier food bio-preservation. Sequential application of mild, mechanistically distinct interventions achieves synergistic microbial inactivation at lower individual doses, with better retention of sensory and nutritional quality.

X. FUTURE PERSPECTIVES

Future developments in bio-preservation represent an important area of ongoing research with significant practical implications, particularly for the Indian food industry. Several directions show substantial promise within the next decade, ranging from near-term technological adaptations to more speculative but scientifically credible innovations.

10.1 Nano-encapsulation of Antimicrobial Compounds

Encapsulating bacteriocins within nano-scale carriers — including liposomes, chitosan nanoparticles, zein protein matrices, and solid lipid nanoparticles — protects them from degradation in complex food matrices and enables controlled release at the site of action over time. This technology is already well established in pharmaceutical drug delivery. Adapting it reliably and safely for food-grade applications at commercial scale is the primary current challenge. For liquid dairy products with extended distribution chains — flavoured milk, lassi, chaas, and UHT beverages — nano-encapsulation of nisin or other bacteriocins could substantially extend bio-preservative efficacy under variable storage and transport conditions (Bhattacharyya et al., 2025). Regulatory approval for nano-scale food additives is still developing in most markets, including India.

10.2 Engineered LAB Strains

Metabolic engineering of LAB strains — to produce higher yields of bacteriocins, to maintain antimicrobial activity under elevated temperatures or extreme pH values, or to target a broader range of pathogens — is an active and well-funded research area. Several recombinant LAB strains have demonstrated significantly enhanced antimicrobial performance in laboratory settings. The regulatory pathway for genetically modified food microorganisms, however, remains a major commercial barrier in most markets. Non-GMO approaches — including directed evolution, adaptive laboratory evolution, and classical strain selection — offer alternative routes to improved performance that avoid both regulatory barriers and consumer acceptance challenges. These approaches may be more practically relevant for the Indian market in the near term.

10.3 CRISPR-Based Antimicrobial Systems

CRISPR-Cas systems can be programmed to recognise and cleave specific nucleotide sequences within target pathogen genomes, achieving a degree of targeting that exceeds even bacteriophage specificity. Delivery via phage vectors makes this approach conceptually deployable in food

processing environments. Whether CRISPR-based antimicrobials can transition from laboratory demonstrations to practical, regulatory-compliant food processing tools remains genuinely uncertain; the path from proof-of-concept to commercial deployment is likely to take decades rather than years. Nevertheless, the early mechanistic evidence is compelling and the area warrants continued research investment (Pujato et al., 2024).

10.4 Intelligent Active Packaging

Packaging materials incorporating bio-preservative agents that respond dynamically to environmental conditions — releasing antimicrobial compounds in response to rising pH, CO2 accumulation, or temperature excursion indicative of microbial activity — represent the most near-term of the emerging applications. For India specifically, where the gap between factory-level food safety standards and the conditions products encounter during distribution and retail is often pronounced, packaging that partially compensates for cold chain failures has direct public health value. Several active packaging systems incorporating nisin, LAB metabolites, and phage preparations are at advanced research or early commercial stages globally. The development of biodegradable active packaging aligned with India's Swachh Bharat sustainability goals adds an additional dimension of policy relevance (Singh & Patil, 2023).

Table 3: Comparative Critical Analysis of Bio-preservative Methods

Method	Spectrum	Speed of Action	Resistance Risk	Consumer Acceptance	Cost
LAB cultures	Broad (acid-based)	Slow–Moderate	Low	Very High	Low
Nisin	Gram-positive only	Moderate	Low–Moderate	High	Moderate
Pediocin / Enterocin	Gram-positive primary	Moderate	Low	High	Moderate
Bacteriophages	Ultra-specific (strain level)	Fast	Very Low	Moderate	Moderate–High
Endolysins	Specific (species level)	Very Fast	Very Low	Moderate	High (currently)

XI. CONCLUSION

Bio-preservation has evolved from an empirically observed phenomenon in traditional food cultures — the dahi pot, the fermented kinema jar, the salt-cured meat — into a scientifically rigorous and increasingly commercially deployed set of technologies. The agents reviewed here span a spectrum from the well-established and globally approved (LAB, nisin) to the scientifically validated but regulatory-pending (pediocin, bacteriophage preparations) to the emerging and high-potential (endolysins, engineered strains). Progress across all categories is substantive, and the mechanistic understanding underpinning each approach is now solid enough to support evidence-based application decisions.

The critical analysis presented in this review underscores that no single bio-preservative agent is universally applicable. LAB cultures act slowly and are strain-dependent; bacteriocins face spectrum limitations and stability challenges in complex matrices; bacteriophages require strain-specific identification and face evolving regulatory landscapes; endolysins are rapid and resistance-resistant but remain cost-constrained. A well-designed multi-hurdle strategy combining two or more of these agents with appropriate non-thermal processing steps represents the most robust and practically deployable approach under current technological and regulatory conditions.

For India, the case for accelerated scientific investment and regulatory development in bio-preservation is compelling on multiple grounds. The food safety challenges created by an inconsistent cold chain, the rapid growth of packaged and ready-to-eat food markets, the expanding urban consumer preference for clean-label products, and the longstanding problem of dairy spoilage all converge on bio-preservation as a practical, scalable, and consumer-aligned solution.

India's traditional fermented food heritage — and the microbiologically diverse, environmentally adapted LAB strains it contains — is an underexplored scientific resource with the potential to yield domestically relevant bio-preservation solutions superior to imported commercial alternatives. This is where some of the most valuable future research effort could be directed: systematic characterisation of indigenous LAB strains, development of FSSAI regulatory pathways for bio-preservative agents, and pilot-scale integration studies in Indian dairy processing environments.

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Data Availability

No datasets were generated or analysed during the current study. This is a review article based on previously published literature.

Competing Interests

The authors declare no competing interests.

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