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Understanding of probiotic origin antimicrobial peptides: a sustainable approach ensuring food safety

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The practice of preserving and adding value to food dates back to over 10,000 BCE, when unintentional microbial-driven chemical reactions imparted flavor and extended the shelf life of fermented foods. The process evolved, and with the urbanization of society, significant shifts in dietary habits emerged, accompanied by sporadic food poisoning incidents. The repercussions of the COVID-19 pandemic have intensified the search for antibiotic alternatives owing to the rise in antibiotic-resistant pathogens, emphasizing the exploration of probiotic-origin antimicrobial peptides to alleviate human microbiome collateral damage. Often termed 'molecular knives', these peptides outstand as potent antimicrobials due to their compatibility with innate microflora, amenability to bioengineering, target specificity, versatility and rapidity in molecular level mode of action. This review centres on bacteriocins sourced from lactic acid bacteria found in ethnic fermented foods, accentuating their desirable attributes, technological applications as nanobiotics and potential future applications in the modern context of ensuring food safety.

Since eons, fermented foods have been a fundamental component of the human diet. In food production, fermentation is a microbially induced intentional preservation method that ultimately results in extending the shelf life of the food and a desirable change in organoleptic properties. Advancements in the fields of biotechnology and nanotechnology have significantly propelled the investigation of fermented foods, including their production, utilisation, and potential health advantages. This area of research has gained substantial momentum due to the widespread prevalence and diverse range of fermented foods in the human diet worldwide. Ethnic fermented foods have been preserved by native communities historically. Thus, such fermented foods have unintentionally undergone a long-term evolution where the native microbial fauna have been preserved. A major contribution to this preservation phenomenon is unequivocally attributed to the resident microorganisms in fermented foods, which ensure food safety by inhibiting undesirable and pathogenic microbes in a natural yet cost-effective manner. Tools like genomics, metagenomics, and other –omics techniques and nanotechnology stepping up as principles behind food preservation to ensure food security.

With the advent of scientific advancement, the antibiotic discovery laid the foundation of modern medicine, which revolutionized the healthcare sector. Antibiotics became the most prescribed medicines in human history. However, their improper use in humans, overuse in both human and agricultural industries, and misuse for non-bacterial infections have paved the way for the evolution of pan-antibiotic-resistant bacterial pathogens. This grim scenario, coupled with fast-paced societal development and globalization, has facilitated the transmission of antibiotic-resistant pathogens worldwide. Consequently, humanity faces a silent pandemic of antimicrobial resistance, AMR[1], which, if left unabated, will jeopardize food security, human life, and the economy. As per estimates by WHO, AMR may lead to a global economic loss of USD 100 trillion, thus pressing the urgency to shift the focus of public health departments towards AMR-borne infections[2]. As per FAO predictions, AMR could push 24 million more people into extreme poverty within the next decade, exacerbating issues of hunger and malnutrition. Moreover, it is anticipated that antibiotic-resistant infections will cause the loss of 10 million lives each year by 2050[3].

In the aftermath of the COVID-19 pandemic, motivated by the issue of nosocomial spread of antibiotic resistance and the desire to minimize the casualties due to incurable infections, researchers have initiated investigations into alternative strategies to classical antibiotics for pathogen control.

Since antimicrobial-resistant bacteria (ARB) are likely to be in contact with food, their transmission has been associated with several foodborne illness outbreaks[4]. This transmission is expected to be more damaging since it points towards the spread of AMR in the continuum[5]. Dissemination of AMR and antimicrobial-resistant genes occurs in various domains, which include agriculture (manure to plants, from phyllosphere pathogens via horizontal gene transfer to soil bacteria), the environment (irrigation

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system, livestock, and aquaculture) and the food processing industries. The latter plays a critical role in transmitting AMR burden from farm to plate, which is likely to have severe repercussions[6].

The above grim situation is further compounded by WHO data that reports that globally an estimated 33 million years of lives are forfeited every year due to ingesting unsafe food[7]. Imperatively, food safety is a vital aspect of ensuring the overall good health of global citizens. As an alternative bio-preservation approach, bacteriocins or bacteriocin-producing starter cultures have gained traction in preserving various food items[8]. Bacteriocins are a class of short peptides that are synthesised by specific bacteria and exhibit a limited range of antibacterial action.

Further, of the few researched options, bacteriocins have emerged as attractive antibiotic alternatives. The non-toxic profile ensuring biosafety and subsequent tremendous industrial applicability set these bacteriocins apart from other available antimicrobials. Though bacteriocins are known to be produced by diverse genera covering pathogenic and non-pathogenic bacteria, this review is centred solely on LAB-origin bacteriocins derived from diverse ethnic fermented foods. The food origin lends the bacteria and their products to be considered under 'generally recognized as safe' (GRAS) status, a designation provided by the United States Food and Drug Administration. A deep insight into an updated classification is provided, and emphasis is placed on its potential for application as an antibiotic alternative as nanobiotic/nanoformulations in food bio-preservation. The review highlights the difference in the mechanism of action of LAB bacteriocin with classical antibiotics, marked antimicrobial potency, microbiome reshaping, and immunomodulatory effect across the gut milieu, and also emphasizes the need for exploration of the role of LAB bacteriocin across the gut-blood and gut-brain axis. Moreover, it delves into the sought-after traits of an ideal bacteriocin, the practical applications of FDA-approved LAB bacteriocins in the market, and confronts potential challenges linked to their utilization to indicate future research scope. The goal of this review is to press upon the need and necessity of Bioengineering from the viewpoint of commercial application of these molecular knives for translation from lab to market, envisioning global food safety.

In a nutshell, we summarize FDA approved GRAS-origin bacteriocin, which is known for its selective inhibition ability and needs to be effectively weighted in the light of disturbance to the microbial biomes that could inflict unwanted health consequences. Thus, this review aims to provide directions towards future research to address multiple facets to ensure global food security, sustainability and circularity.

Bacteriocin sourced from fermented foods

Ethnic fermented foods and beverages have played a pivotal role in human diets. The earliest accepted evidence for fermented food (kefir) goes as back as 3000 to 5000 years ago, corresponding to the Bronze Age[9]. Thus, beneficial bacterial interactions with humans consciously have been going on for a considerable period of time. The strains have evolved during this period, while ethnic communities have preserved these strains via constant 'unintended' selection. Fermented foods are the product of the oldest biotechnological processes that promote gut microbiota health, as depicted in Fig. 1. The fermentation phenomenon practiced by humans dates back to 13,000 BC, which has slowly become an integral part of the cultural heritage[10]. This old biological process was employed to extend the storage duration, enhance shelf life, and maintain the organoleptic properties of food products by exploiting the naturally occurring microbes of food[11,12]. The fermentation of milk occurred by chance and not by design which initiated one of the longest microbially assisted food evolution experiments, still practiced by traditional and ethnic communities.

Modern biological tools are currently being applied to reap the benefit of these traditional fermented foods to ensure food safety, nutrition, and sustainability. Ethnic fermented food encompasses a rich probiotic content, out of which LAB are most common. Besides their potential application as

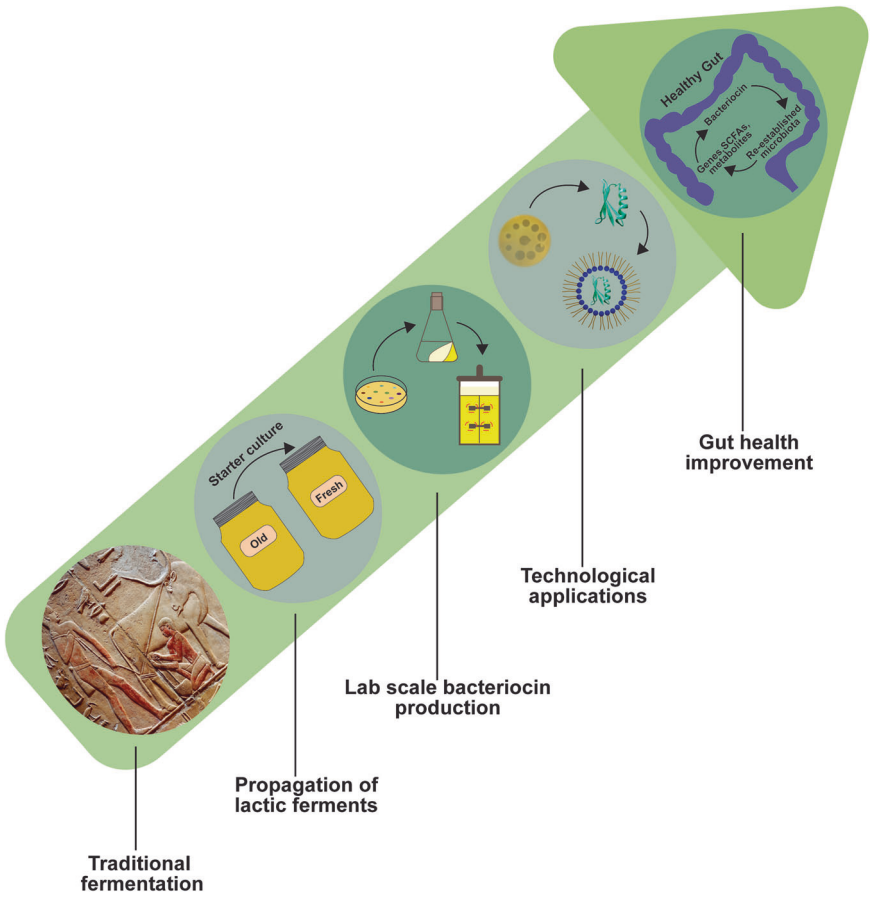


Fig. 1 | Hierarchical link between traditional fermentation and gut engineering. The ethnic fermentation practices lead to the propagation of lactic ferments as well as the production of bacteriocin, which can be technologically advanced and engineered for enhanced metabolite production and healthy gut microbiota.

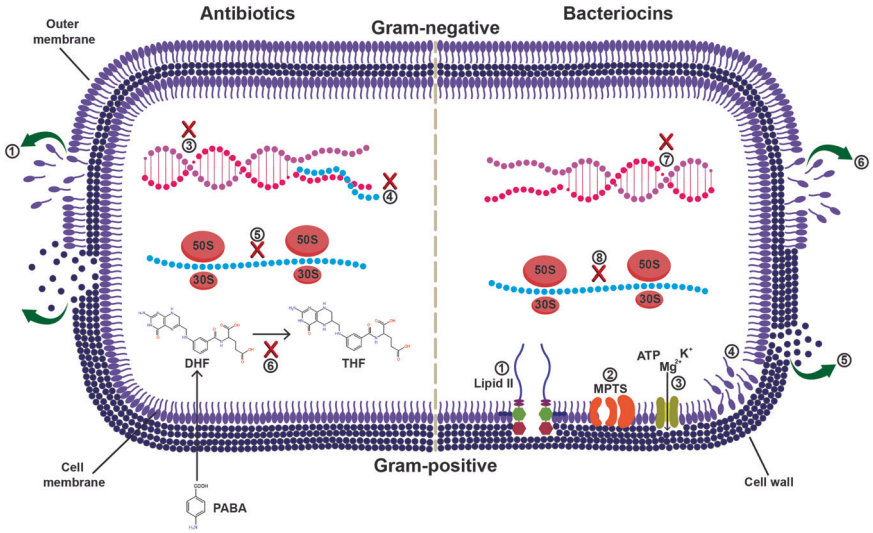


Fig. 2 | Mechanism of action of different classes of antibiotics and bacteriocins. In both Gram-positive and Gram-negative bacteria, antibiotics and bacteriocins execute antibacterial actions via variety of processes, including the following: 1. Disintegration of outer membrane 2. Cell wall biosynthesis inhibition 3. Inhibition of DNA replication 4. Transcription 5. Protein synthesis 6. Folate biosynthesis and LAB bacteriocins: 1. Membrane disruption through pore formation after binding to lipid II, a peptidoglycan precursor (Nisin, Lacticin) 2. Pore formation by binding to the mannose phosphotransferase system (Pediocin, Garviecin) 3. Consequent release of ATP and ions on PMF disturbance 4. Disruption of cell membrane (enterolysin A) 5. cell wall 6. Outer membrane (Helveticin, lytic class III bacteriocin) 7. Inhibition of DNA replication 8. Protein synthesis (Caseicin, non-lytic class III bacteriocin) in gram-positive and gram-negative bacteria.

probiotics, they are often screened for producing antimicrobial peptides. LABs possess a qualified presumption of safety (QPS) status; therefore, bacteriocins produced by them are regarded as safe by the US FDA (The U.S. Food and Drug Administration)[13]. Several reports of bacteriocin producing LAB isolated from fermented foods are available in extant literature since traditional foods harbour various microbial populations producing different secondary metabolites[14–17]. The spectrum of activity of the bacteriocins varies from strain to strain, providing a scope to study fermented foods belonging to diverse ethnicities. Apart from the association with the food grade, bacteriocins produced by LABs are advantageous due to versatility in mode of action and structure, owing to the diversity in the gene coding for synthesis, immunity, and secretion[8]. Extensive studies on bacteriocins have established food-preserving characteristics and their effectiveness as health modulators, besides their renowned functionality as antimicrobials. These antimicrobial peptides are gaining a lot of consumers' attention due to their prolonged historical acceptance, utilization, and activity at nanomolar concentrations; hence, they act as the natural substitute for antibiotics in the current scenario of increasing antimicrobial resistance and related threats.

An updated classification of Bacteriocin: a natural substitute to classical antibiotics

Antibiotics have played a pivotal role in facilitating remarkable advancements in both medical and societal domains, thereby establishing themselves as the most commonly prescribed medications in contemporary society. However, the widespread and uncontrolled usage of antibiotics, along with the scarcity of innovative therapeutic options being developed, has led to considerable escalation in the AMR crisis. Consequently, global inhabitants are now on the brink of entering a post-antibiotic era. This, in turn, is anticipated to lead to the emergence of a silent pandemic involving antibiotic-resistant microbes in the near future. Further, resistant bacteria have been linked and disseminated via food products[18]. Hence, there is an urgent need to address the issue of antibiotic-resistant pathogens, primarily in the food and health sector. The concept of "one-health" addresses these issues considering the whole ecosystem. Given the potential harm broad-spectrum drugs can cause to the beneficial gut microbiota in humans, several efforts have been made to explore novel therapeutic molecules with distinct modes of action. Furthermore, to address concerns related to the diminishing effectiveness of various antibiotics, the built up of antibiotic residues in the environment and food chain, the prevention of resistance gene transfer, and the assurance of food safety and quality, it has become increasingly imperative to identify viable alternatives to conventional antibiotics[19].

Bacteriocins, also called antimicrobial peptides (AMPs), are bacterial peptides synthesized within ribosomes and categorized as potential therapeutic agents owing to their potency against infectious pathogens[20]. The rapid mechanism of action of these gene-encoded peptides makes it challenging for the pathogenic bacteria to acquire resistance against bacteriocins[11,21]. Due to its potential as a natural food preservative and as a possible class of therapeutic antibiotic for human use, bacteriocin research, particularly the ones derived from LAB, has garnered significant attention and value in the last decade, especially after successful approval of nisin, pediocin PA-1/AcH for use as bio-preservative (food safety) and in medical applications against antibiotic-resistant strains (VRE, MRSA) and known food pathogen, *L.monocytogens*[22].

Antibiotic and LAB bacteriocins vary in their respective mechanism of action (see Fig. 2) despite certain similarities, like the target that they act upon (vancomycin and nisin share the same target, i.e. lipid II)[23]. The molecular-level mode of action of antibiotics varies based on their class. For example, β-Lactam antibiotics impede the biosynthesis of the bacterial cell wall, Quinolones constrain DNA replication, Aminoglycosides and tetracycline target the 30S subunit of the ribosome, thereby halting protein biosynthesis, and Sulfa drugs impede the biosynthetic pathway of folate coenzymes[24]. However, bacteriocins primarily exert their antimicrobial action by interacting electrostatically with the target cell membrane, causing structural damage and the subsequent release of cellular contents. Lantibiotics I and II, exemplified by nisin A and lacticin 3417, induce membrane disruption through pore formation after binding to lipid II, a peptidoglycan precursor on the cell membrane[25]. Pore formation disrupts the proton motive force (PMF), resulting in the accumulation of ions/metabolites on the membrane[20], thereby impeding energy-intensive chemical reactions within the cell[26]. Furthermore, certain Class I bacteriocins, like nisin, have been observed to exert supplementary effects on target cells. These effects include the separation of endogenous autolysins from their natural inhibitors, leading to cell lysis, as well as reactive hydroxyl radicals accumulation that contribute to antibacterial activity[27]. In contrast, class IIa bacteriocins create pores by binding to the mannose phosphotransferase system[28]. Bacteriocins categorized in this group demonstrate unique mechanisms of action. Some of these bacteriocins adhere to the Mannose-phosphotransferase system, while others disrupt the synthesis of the target cell's cell wall, leading to eventual cell death[29]. Class III bacteriocins have received comparatively less attention in terms of mode of action. Unlike other classes where the primary target is the cell membrane, Class III bacteriocins focus on the cell wall. Their action mechanism appears to be either bacteriolytic or non-lytic[30]. Non-lytic class III bacteriocins, such as caseicin from *Lactobacillus casei*, inhibit DNA and protein synthesis without causing cell lysis. Helveticin M (lytic class III bacteriocin), exerts its antimicrobial activity by disrupting the cell wall and outer membrane of gram positive and gram-negative bacteria respectively, thus displaying its effectiveness as

broad-spectrum antimicrobial[30]. Class IV bacteriocins like enterolysin A inhibit cell growth by forming circular structures in a head-to-tail manner[31], displaying broad-spectrum antimicrobial activity.

Thus, increased attention to bacteriocins from 'safe' sources is attributed to their dual potential as therapeutic antimicrobial agents and natural food preservatives, which holds significant relevance in today's context. American Food and Drug Agency (FDA) has granted GRAS status to LAB due to their historical use in ethnic fermented foods, and the European Food Safety Authority (EFSA) has conferred Qualified Presumption of Safety (QPS) status to various LAB genera viz. *Leuconostoc*, *Lactobacillus*, *streptococcus* and *Pediococcus*, respectively[32].

Besides bacteriocins, LABs are known for producing lactic acid (as a by-product of food fermentations) and antimicrobial substances like acetoin, hydrogen peroxide and short-chain fatty acids[33,34]. Bacteriocins from LAB possess several favourable characteristics that have rendered them appealing for a number of applications. They exhibit an inherent tolerance to elevated thermal stress, rapid activity at minimal concentration, substantial potential at the nanoscale, effective functionality over a broad pH spectrum, and, crucially, offer flexibility for genetic manipulation due to their elementary biosynthetic mechanisms[11,35]. Their distinguishing attributes, including their colorless, odorless, and tasteless nature, render them highly suitable for use as food preservatives, further enhancing their applicability[36]. Susceptibility towards digestive enzymes like trypsin, pancreatin complex, and chymotrypsin makes them a safe choice among antimicrobials for the gut since they do not exert any detrimental effect on resident microbiota of the gut or elicit immune response[34]. Bacteriocins' remarkable trait is their ability to selectively target both related and unrelated bacteria, excluding the producer cell itself. This is achieved by synthesizing self-immunity proteins, which safeguard the producer cell by either scavenging the bacteriocin or engaging in antagonistic competition for the bacteriocin receptor[19]. Besides the adverse effects associated with antibiotics, the recent emergence of multidrug and extensively drug-resistant strains (MDR and XDR) has presented a pressing concern[37]. The high target specificity of bacteriocins, even against MDRs, positions them as a potent and ideal candidate for use in clinical and food-related applications[23]. Due to the well-known role of LAB in the fermentation phenomenon dating back to ancient times, bacteriocins sourced from them are deemed food-grade and, thus, carry the advantage over traditionally used antibiotics in the market[11]. After the proposal of the first classification of LAB bacteriocins back in 1993[38], researchers kept updating the classification of bacteriocins to accommodate the subsequent new classes or subclasses being discovered with time. Most of them are in agreement with proposals previously introduced by ref. 39. According to this, the bacteriocins were divided into three categories depending on their mode of action and structural attributes. Bacteriocins containing lanthionine were referred to as class I, which exhibits a dual mode of activity by either binding to lipid II, transporter of peptidoglycan unit, thus inhibiting cell wall synthesis or by considering the former a docking molecule followed by pore formation and cell death. In comparison, non-lanthionine-containing bacteriocins constituted class II, further divided into 5 other subclasses. The bacteriocins in this category were described as carrying a helical structure (amphiphilic), facilitating the insertion into the cell membrane leading to depolarisation and, subsequently, cell death. Lastly, this system categorized non-bacteriocin lytic proteins in the third class, 'Bacteriolysins'.These include all heat labile, large murein hydrolases which directly target the gram-positive cell wall and lead to itslysis. Moreover, they reserved the class IV category for those bacteriocins that need a non-proteinaceous moiety for their antibacterial activity; however, it was not formally included as an individual class since none of its members have been explored yet[39]. Finally, circular LAB bacteriocins were proposed to be categorized under class V[40].

Recently, another classification system was introduced, which proposed to divide the bacteriocins according to the origin of synthesis and molecular weight[13]. As per this system, class I comprises peptides with size <10 KDa, synthesized ribosomally and modified post-translationally, termed Ripps, which carry molecules with amino acids like lanthionine. In contrast, unmodified peptides were placed in class II, carrying a leader peptide for mediating translocation. The third class of bacteriocins was allocated to bacteriocins with bacteriolytic or non-bacteriolytic activity with >10KDa molecular weight[13]. Another recent classification scheme distributed the bacteriocins into 4 classes, where class I denotes small peptides with <5KDa size representing lantibiotics, Class II encompass non-lantibiotics having size < 10 kDa while large peptides (lytic or non-lytic) were allocated to Class III, which were heat labile. Lastly, class IV comprises lipids or carbohydrate parts, basically non-protein bacteriocin candidates, which kill the target cell by disrupting the cell membrane[41]. Recently, an updated system has been presented wherein, according to the type of bacteria (Gram-positive and gram-negative), bacteriocin has been classified into two different classes, i.e. modified (class I), having molecular mass < 5 kDa and unmodified (class II) with 6–8 kDa size.

In accordance with the RiPP nomenclature, the subsequent subcategorization of class I aligns with the classification proposed by ref. 23. Another classification system excluded leaderless bacteriocins as a subclass of Class II but was further divided into three subclasses, namely, pediocin-like, comprising of a consensus sequence (YGNGV), non-pediocin bacteriocin, which does not contain this characteristic motif and two-peptide bacteriocins respectively[13]. The comprehensive analysis of genome mining with reference to LAB reveals that the range of antimicrobials encoded in public sequences might exceed initial expectations. This includes certain putative classes like lasso peptides and sactipeptides, which have not been reported in LAB until now, indicating extensive possibilities for future applications[13]. Table 1 provides an overview of the antimicrobial activity of different bacteriocins produced by LAB isolated from traditional fermented foods.

Commercial application

Consistently increasing customer awareness, preference, and demand for bio preservatives in the place of chemical preservatives in food industries have spurred extensive research for broad-spectrum antimicrobial peptides from LAB sourced from fermented foods for application in food systems. The number of patents awarded for bacteriocin technology is on the rise, driven by the growing need for these compounds in food matrices and medicines coupled with biotechnological advancements. The United States has received 42% of all awarded patents, followed by China at 12% and Denmark at 10%, according to recent research[42]. Depending on the rules, regulations and intended use in individual countries, the legislation pertaining to the utilization of bacteriocins in food products differs[19]. Nisin became the first bacteriocin that was added to the European food additive list and received FDA approval in 2017[43]. Stringent rules and regulations by health regulatory bodies and the cost incurred in bacteriocin isolation and downstream processing are other reasons attributed to restricted commercial exploration and approval of various bacteriocins[44]. Many bacteriocins are still awaiting legal approval for commercialization as biopreservatives, namely, enterocin AS-48 lacticin 3147[13]. Table 2 displays some of the commercially approved bacteriocins for food preservation. Besides bacteriocins, the producer cells, referred to as protective cultures, are also employed for food preservation or as additives like *Carnobacterium divergens* M35 for use in sliced cold-smoked salmon (ready to eat product); cold-smoked trout, *Carnobacterium maltaromaticum* CB1, *Leuconostoc carnosum* 4010 (Danisco, HOLDBAC®) (Vacuum-packed bologna; cervelat; frankfurters; mortadella; wieners) after receiving approval from respective country's food safety authorities (for instance Health Canada)[19].

LAB bacteriocin as an immunomodulator and antimicrobial agent

The impact of the human microbiome on overall health is substantial, with implications for a wide spectrum of conditions, including everything from acute gastrointestinal infections to chronic problems like joint pain, metabolic disorders, and persistent disorders like neurodegenerative diseases[45]. Consequently, there has been a significant focus on investigating the relationship between microbiome patterns and various diseases to gain insights

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Table 1 | Classification of bacteriocins from LABs – Class I, Class II, and Class III types in a tabulated format

Bacteriocin producer strain	Bacteriocin	Class	Fermented food	Spectrum	References
Lactobacillus plantarum J23	Lac-B23	Class II	Chinese traditional fermented milk	*Listeria monocytogenes*	123
E.italicus ONU547	Bacteriocin ONU547	Class I	Thai fermented cabbage	*L. sakei subsp. sakei* JCM 1157	17
P. pentosaceus 63, *P. pentosaceus* 145, *P. pentosaceus* 146, and *P. pentosaceus* 147	Pediocin PA-1	Class II	Minas cheese	*L. monocytogenes*	124
Lactobacillus sakei	Y19-2	Class III	Nanjing Steamed Roast Duck, Chinese fermented meat	Broad spectrum	125
L. curvatus LAB-3H	Bacteriocin	Class III	Traditional yoghurt	*L. monocytogenes*, *E. coli*, *Bacillus cereus*, *S. aureus*	15
Lactobacillus plantarum MXG-68	Plantaricin MXG-68	Class II	Inner Mongolia traditional fermented koumiss	*S. typhimurium* ATCC14028	126
Lactobacillus plantarum KIBGE-IB45	Bac-IB45	Class III	Cheddar Cheese	*Listeria monocytogenes* ATCC 7644	16
Lactobacillus fermentum BZ532	LF-BZ532	Class I	Chinese Bozai "fermented cereal"	*Escherichia coli* k12, *staphylococcus aureus* ATCC6538 *and Salmonella sp.* D104	14
Lactobacillus paracasei LS-6	LSX01	Class I	Yunnan traditional fermented yoghurt	*S. aureus*_45	127

Table 2 | Commercially approved LAB bacteriocins for food preservation

Bacteriocin	Producer organism	Commercial name	Application in Food industries	Target organism	References
Nisin Z, Nisin A	*Lactococcus lactis* isolated from Sauerkraut, *Lactococcus lactis*	Nisaplin™ (Danisco, E234), Nisin A® Nisin Z® (Handary, Brussels, Belgium)	Meat, bakery products, beverages, culinary, and dairy products	*Bacillus spp. Clostridium spp. Listeria spp.B.cereus*	39
Nisin	*Lactococcus lactis*	Chrisin® (Chris Hansen, Horsholm, Denmark), Delvo®Nis (DSM, Delft, Netherlands), Biosafe™ (Chr. Hansen)	Meat, sausages, Dairy industry, processed hard and semi-hard Cheese	*Listeria monocytogenes, Clostridium botulinum*, *Bacillus cereus* and *Listeria monocytogenes, Clostridium spp*. Present in milk	19,43
Pediocin PA-1	*Pediococcus acidilactici*	Alta 2341™ (Concentrated fermentate) (Quest International, Irvine, CA, USA)	Ready-to-eat meat products,	*Listeria monocytogenes*	128
Pediocin PA-1	*Pediococcus acidilactici*	MicroGARDTM (Bioactive powder) (DuPont)	Seafood, Meats, Poultry, & Alternatives	*L. monocytogenes*	26,44
Pediocin PA-1	*Pediococcus acidilactici*	DuraFreshTM UC (Kerry), a blend of blend of organic acid and peptide	Poultry and meat preservation	*Clostridium perfringens, Listeria monocytogenes*, *Clostridium botulinum*	19
Sakacin,Pediocin	*Pediococcus pentosaceous, Lactobacillus sake*	Bactoferm F-LC®(Chr. Hansen, Hørsholm, Denmark)	Meat products	*Listeria monocytogenes*	129
Sakacin,	*Lactobacillus sake*	Bactoferm™ B-2, Bactoferm™ B-FM	Meat products	*Listeria monocytogenes*	130
Leococin A	*Leuconostoc lacti*	Bactoferm™ B-SF-43	Meat products	*Listeria monocytogenes*	130
Pediocin	*Pediococcus pentosaceous*	Fargo 23°(Quest International, B.V., Naarden, The Netherlands)	Accepted for commercial use in meat products	*Listeria monocytogenes*	131
Micocin	*Carnobacterium maltaromaticum* UAL307	Micocin®,(CanBiocin, Edmonton, Canada)	Meat products	*Listeria monocytogenes*	42

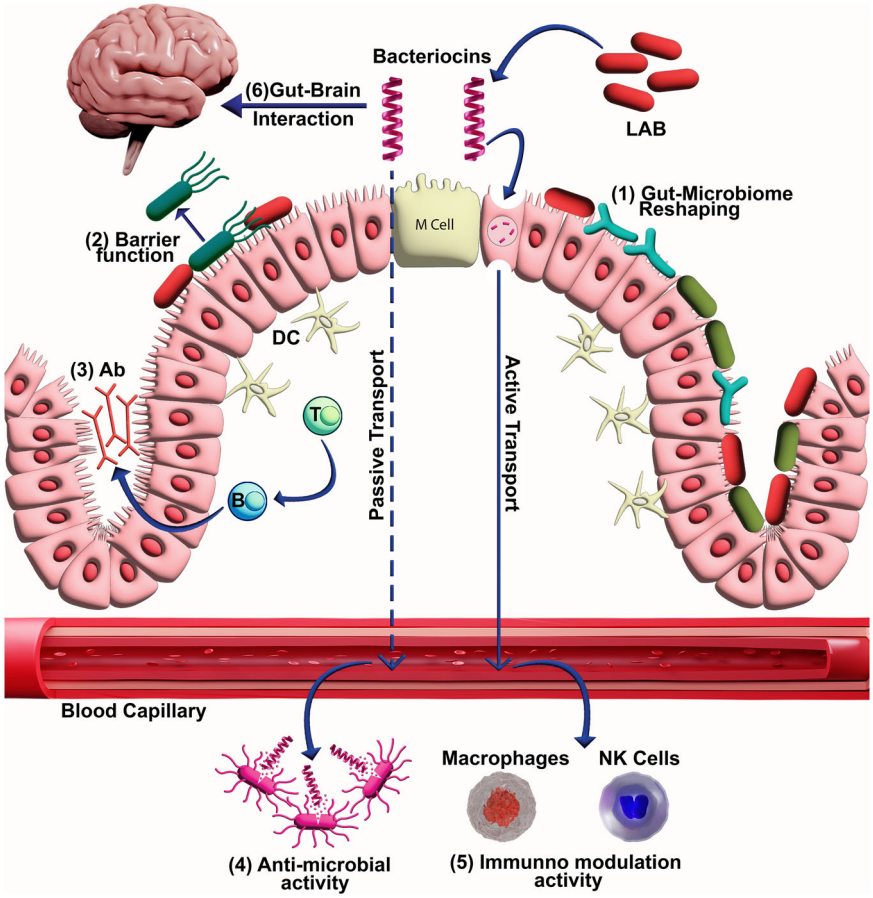


Fig. 3 | Schematic representation of bacteriocin activity in the gut. Bacteriocin is responsible for mediating various functions across the gastro-intestinal and vascular barrier, which include 1. Gut-microbiome reshaping, 2. Enhanced barrier function, 3. Antibody production, 4. Antimicrobial activity, 5. Modulation of innate and adaptive immunity by activation of DC, NK cells and macrophages, 6. Bacteriocin cross-talk across gut-brain axis. NK cells: Natural killer cells, DC Dendritic cells, Ab Antibodies, LAB Lactic acid bacteria, T Thymus, B Bone marrow. Blue solid line- Active transport, Blue dashed line- Passive transport.

into microbiome dynamics, the mechanisms that influence bacterial competition, and the modulation of microbiome-host communication[46]. Besides, LAB bacteriocins need in depth understanding and exploration in terms of in vivo and in vitro efficacy to ensure their potent antimicrobial potency for application in preservation of functional foods.

Role of LAB bacteriocins across gut milieu and gut-brain axis

Antimicrobial peptides play a pivotal role in modulating the host native microbiota and have been reported to ameliorate dysbiosis, thus improving overall gut and host health.

Recent research on orally administered nisin indicates that it remains structurally intact as it traverses the porcine gastrointestinal tract. This was confirmed by assessing both its molecular weight and its activity. Nisin exerts a notable influence on the overall composition and function of the gut microbiota, resulting in a reversible reduction in the population of gram-positive bacteria (which includes reshaping of the Firmicutes) and a reversible enhancement in the population of gram-negative Proteobacteria. These modifications, in turn, lead to changes in the abundance of pathways responsible for producing short-chain fatty acids (SCFAs). For instance, there is a decrease in the synthesis of acetate and butyrate but an increase in propionate production. Consequently, there is a reduction in the levels of SCFAs in faecal matter. In summary, bacteriocin, such as nisin, appears to play a pivotal role in reshaping mammalian microbiomes (dynamics and composition) and influencing the overall functionality of the microbial community, as noted in the study by ref. 47. Colicin Ib, a pore-forming bacteriocin, enterocin 21, microcin M, and nisin A have been documented to impede the colonization of pathogenic bacteria as demonstrated via resistance assays[46]. Besides antimicrobial activity, bacteriocins are also known to exhibit cytotoxic effects on host cells. Reports indicate the role of cytolysin from *E.faecalis and* streptolysins from *Streptococcus pyogenes* on a wide range of host cells, viz. neutrophils, erythrocytes and macrophages, respectively[48,49]. The biosynthetic gene clusters (BGCs) responsible for bacteriocin production undergo lateral transfer and recombination, which can result in various consequences for the recipient cell, including the potential to modulate immunity, target range, and bacteriocin production. Incorporating a newly acquired bacteriocin can have adverse effects if it hampers a strain that provides vital nutrients to the bacteriocin-producing organism. A bacteriocin imparts a notable competitive edge only when it suppresses competing rival strains without causing harm to mutually beneficial partners[50]. Bacteriocin can exert diverse influences on an existing microbial community, either fostering or impeding the introduction of a new bacterial community or causing a rearrangement of the microbiome. Conversely, it can also enable an invading strain to outcompete the native bacteriocin-sensitive community. Resident bacteriocin-producing strains can act as a barrier against the colonization of specific antibiotic-resistant pathogens or bacteriocin-sensitive newcomers[46].

Furthermore, considering their antibacterial characteristics, certain bacteriocins exhibit other effects impacting the resident microbiome and the host (see Fig. 3). Moreover, Nisin possesses the potential to reduce proinflammatory cytokine levels in human blood cells, thereby influencing the innate[51] and adaptive immune response[52]. According to an in vitro study, nisin has the potential to activate polymorphonuclear neutrophils wherein exposure of neutrophils to nisin in Jurkat and Molt-4 cells significantly elevated intracellular superoxide levels[52]. In response to bacterial lipopolysaccharides in human peripheral blood mononuclear cells, Nisin Z stimulated the secretion of chemokines MCP-1, IL-8, and Gro-a, while drastically reducing the activation of TNF-α, thus, modulating host immunity[53]. In a similar study, nisin modulated the expression of inflammatory response markers such as N-acetyl-β-d-glucosaminidase and TNF-α in BME-UV1 epithelial cell lines[54]. Nisin has been proven to exhibit an in vivo immunomodulatory impact, specifically by increasing the levels of $CD4^+$ and $CD8^+$ T lymphocytes while reducing levels of B lymphocytes.

Prolonged exposure to nisin brings about a balance in the levels of B and T lymphocytes.

The potential of plantaricin NC8 αβ has been established in enhancing keratinocyte's inflammatory responses post *S. aureus* infection while simultaneously reducing proinflammatory cytokines[55]. In another in vivo study, palntiracin PlnEFI has been demonstrated for its ability to reduce inflammation in a model of acute colitis in mice[56]. Immunomodulatory effects of Enterocin OE 342 has also been investigated which indicated downregulation of TNF-α, modulation of IFN-γ and IgA[57,58]. A Recent study have revealed an upsurge in pro-inflammatory cytokines by Salivaricin LHM in in vitro and in vivo models indicating bacteriocin's potential application as a potent antibiotic substitute[59].

Some bacteriocins (modulin) can stimulate proinflammatory responses, resulting in harm to host cells, while others, called siderophore bacteriocins, contribute to the regulation of transition metal levels in bacterial cells[60] or facilitate communication between different bacterial species[61].

A small number of studies have shed light on how bacteriocins behave across gastrointestinal and vascular barriers, but there is a dearth of data regarding their stability in the GI tract. Therefore, additional in-depth studies are needed to completely understand the time and processes involved[62,63]. The results of a recent study revealed that out of three different compounds tested, plantaracin 423, nisin, and bacST4SA, the former two are the most effective at migrating across monolayers of Caco-2 and HUVEC (human umbilical vein endothelial cell). Nevertheless, nisin exhibits a higher affinity for or penetrates cells more effectively than plantaricin 423[64]. The work provides evidence that bacteriocins have the ability, at least in vitro, to traverse the gut-vascular barrier. Paracellular transport may explain why no cytotoxicity was noted at effective blood plasma concentrations of bacteriocin; nonetheless, to determine whether or not bacteriocin is effective in bridging the GI or gut-blood barrier under extreme circumstances, in vivo research is prioritised.

The precise function of bacteriocins or their producers needs to be better established about the gut-brain axis. Extant studies have not provided direct proof to suggest their involvement in these complex interactions[65]. In one of the unique studies, the effects of nisin on aquaporin, a well-known neurotransmitter and commensal gut microbiota were analysed via high-throughput sequencing in an in-silico analysis, which indicated a relationship between the two. The highest expression of norepinephrine in the brain was brought about by nisin in comparison to the untreated group and antibiotic (ciprofloxacin) treated group. Furthermore, the in vivo study conducted on mice demonstrated an elevation in the population of *Bacteroides*, *Lactobacillus* and *Bifidobacterium spp*., along with a reduction in gut pathogens in the caecum sample. This study indicates a robust relation between the reduction in stress evoked by *E. coli*, gut microbiota and nisin in the mice model[65]. Thus, studies targeting the role of the precise function of bacteriocins or LAB that produces them in the gut-brain axis are the need of the hour.

Summary of in-vitro and in-vivo studies on antibacterial potency

Bacteriocins, whether naturally occurring or synthetically produced, have undergone extensive evaluation through in vitro experiments, which include assessment of their antibacterial effect, haemolytic activity, and cytotoxicity on human cell lines. While in vivo studies for toxicity and safety in animal models such as murine[66] and pig models have also been accomplished, as demonstrated by[67]. Additionally, alternative models like zebrafish embryos, C. elegans, and D. melanogaster, as described in the works of[68–70] have been utilized. It is worth noting that comprehensive in vitro and in vivo investigations are scarce for bacteriocins isolated from probiotic bacteria sourced from traditional fermented foods, which warrants further research.

Recently, bacteriocin CH3 was isolated from *L. lactis* CH3 (MZ636710) sourced from fermented dairy products and its antibacterial potential under various physicochemical conditions, like varied pH range, temperature, detergents, organic solvents, and enzymes were assessed. The in vitro antimicrobial activity results indicated effective inhibition of both Gram-positive and Gram-negative human pathogens, specifically *S. aureus*, *K. pneumoniae*, and fungus *C. albicans*. Bacteriocin treatment led to structural deformities and disintegration of the cell membranes of the pathogens. Moreover, the free radical scavenging capability of CH3 was evaluated using 1, 1-diphenyl 1–2-picrylhydrazyl, DPPH assay demonstrated a higher capacity for scavenging radicals, as indicated by an EC50 value of 12.5 µg/ml. The toxicity profile of bacteriocin was validated, and notably, CH3 exhibited minimal toxicity towards dermal fibroblast cells of humans (83.2% at 100 µg/ mL), indicating high possibilities of CH3 being used as a safer biomedicine[71].

The potential antibacterial efficacy of antimicrobial peptides from fermented bovine colostrum whey utilising the proteolytic enzymes of *L. rhamnosus* C25 was evaluated, along with their capacity to boost antibacterial activity against drug-resistant bacteria. The bioactive peptide fractions were examined for their antibacterial potency against a drug resistant *E. coli* strain (ESBL 1384), *E. coli* ATCC25922, *A.baumannii* strains (ATCC 17978, 1379) and *S. aureus* (MRSA 1418, MTCC1144). In addition, the checkerboard approach was employed to investigate the potential synergistic impact of peptides in combination with conventional antibiotics. The cooperative effect of peptide fractions having molecular weight below 10 kDa, combined with specific antibiotics (Levofloxacin, Ciprofloxacin, Chloramphenicol and Rifampicin), was substantiated by ΣFICI. This demonstrated synergistic behaviour against the drug-resistant target ESBL 1384 strain, while *Acinetobacter* 1379 and an MRSA strain resisted the antibacterial effect of the synergists. Furthermore, cytotoxicity assessment was done using an MTT assay on the HT-29 cell line, exhibiting no adverse effects from either peptide fraction[72]. Besides bacteriocin alone, their conjugates with nanoparticles are also employed for various applications, such as antimicrobial protective films for food products or antimicrobial sprays to check post-harvest losses. In a recent investigation, a nanobitoic platform was generated wherein the antibacterial potency of pediocin-silver nanoparticle conjugate was harnessed to mitigate pathogens which are resistant to multiple drugs, including ESKAPE category[21]. The conjugate's in vitro and in vivo efficacy was assessed, Pd-SNPs showed cytocompatibility with NIH 3T3 and HEK293 cells, exhibiting no haemolysis and minimal cytotoxicity at minimum inhibitory concentrations. At 50 times the MIC (*V. fluvialis* L-15318), high cell viability was maintained, indicating compatibility with mammalian cells and red blood cells. In vivo, Pd-SNPs were tested in a VISA-ST1745 Infection Mouse Model at doses of 1–10 mg/kg. No toxicity was observed, and various physiological parameters remained stable, demonstrating the safety of the nanoconjugate across different dosages[21].

Potential application of bacteriocin as nanobioconjugates

For overall performance enhancement of LAB bacteriocins, certain technologies like hurdle technology (an integrated approach comprising of chemical, physical and biological food preserving methodologies), embedding (immobilization methodologies to confine bacteriocin) and collaborative technologies (combination of hurdle, embedding technologies) should be implemented[73].

Bacteriocin from LABs are gaining credibility for their development as nanobiotic platforms. There is a dearth of potent antimicrobials that can target a broad spectrum of pathogens in the food system. Recent developments in lantibiotics from lactobacilli and their decoration on nanoparticles targeting food safety have fuelled much interest among researchers. One of the constraints of bacteriocins is their limited antimicrobial activity on related organisms only. Since most food-grade bacteriocins were discovered from gram-positive bacteria, their activity was also directed against the same. However, approximately 69% of food-borne illnesses are caused by Gram-negative bacterial pathogens[74]. Thus, leaving an unmet need for developing bacteriocin-based strategies to mitigate Gram-negative pathogens associated with food spoilage. Researchers have successfully tried advances in the nanotechnology field to either decorate bacteriocins on nanoparticles or encapsulate nanoparticles in the core-shell of various food-grade materials (see Fig. 4). Bacteriocin-nanoconjugates emerged as an excellent approach to combat the challenges posed by bacteriocin's limited

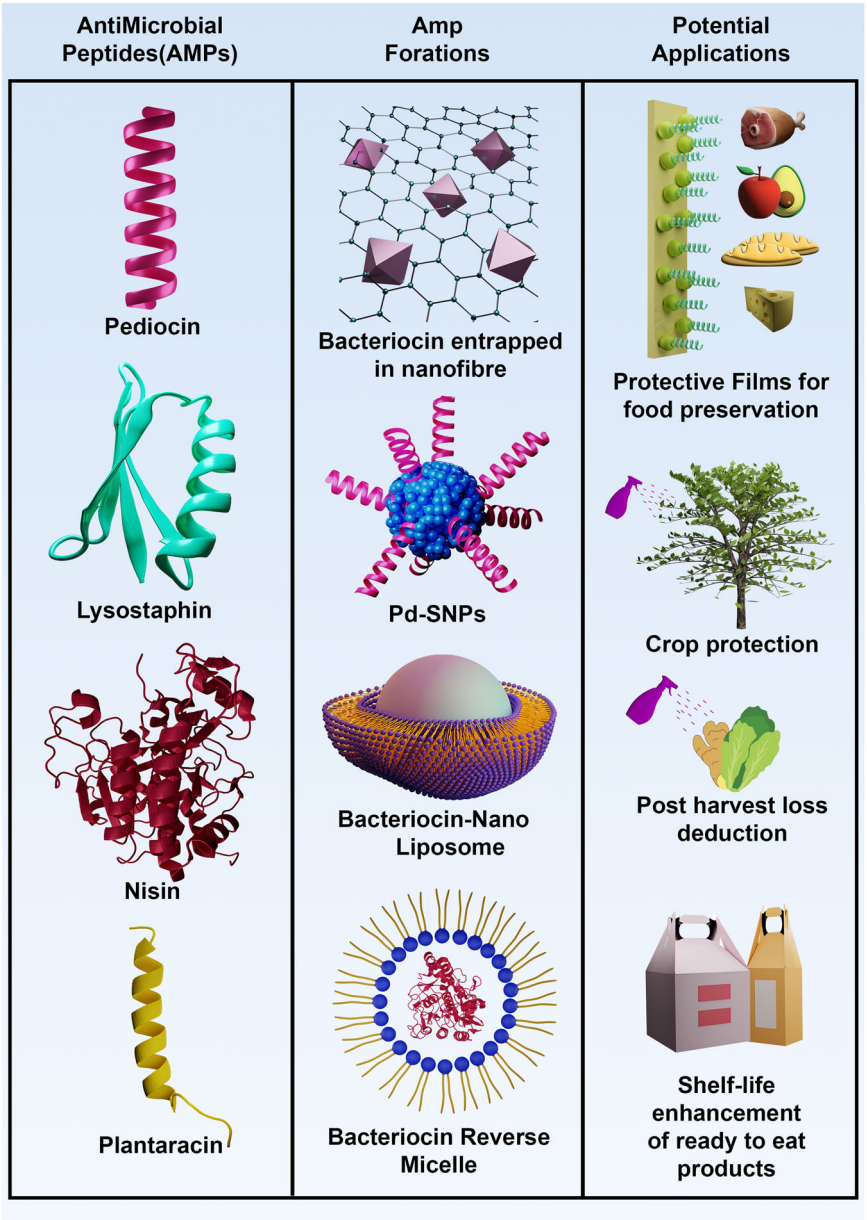


Fig. 4 | Illustrates different bacteriocins, their diverse nanoformations, and their respective applications in the context of food safety. Bacteriocins have the potential to be transformed into nanobiotics through the application of nanotechnology advances that are aimed at ensuring food safety vis-à-vis perishable food products and horticultural crops. The structures of Lysostaphin, Nisin and Plantaracin were adapted from PDB, and the PDB IDs are 4LXC[138], 4MZD[139], and 2JUI[140], respectively.

antimicrobial efficacy and the need for high doses[75]. This was accomplished by decorating bacteriocins over metal nanoparticles to enhance the activity of bacteriocin and widen their antimicrobial spectrum, which eventually led to expedited research in the development of several nanobiotic platforms.

Many studies have employed LAB bacteriocin isolated from raw milk (Bac4463, Bac22, Bac 23) capped silver nanoparticles against food spoilage bacteria, viz. *Shigella flexneri, S. aureus* and *P. aeruginosa*, respectively. The findings have demonstrated improved in vitro efficacy of nano-biotics compared to bacteriocins alone, suggesting the potential use of bacteriocin-capped nanoparticles in food preservation[76].

Bacteriocin conjugates, including enterocin and nisin, have been studied with silver and gold nanoparticles, to target microorganisms responsible for food spoilage[77,78]. Additionally, other studies have explored using chitosan/alginate nanoparticles in conjunction with bacteriocins to combat specific microbes, such as *E. coli, and S. aureus*[79,80]. The toxicity of metals in nanoform to the mammalian cells has remained an issue for bacteriocin-decorated nanoparticles incorporation into the food matrix directly; hence, the nano-conjugated forms of bacteriocins were proposed for food packaging materials initially. However, the synergistic mode of action revealed that the amount of metal nanoparticles required in some specific cases was well below the toxicity limit for mammalian cells. This observation was also confirmed with in-vivo studies using a rodent model[21].

Synergistic antibacterial activity of a food-grade class IIa bacteriocin, pediocin (isolated from *Pediococcus pentosaceus* sourced from cheese) with silver (PD-SNPs) was explored, and pediocin-coated silver nanoparticles were developed to mitigate multidrug-resistant bacterial pathogens including ESKAPE category. The developed Pd-SNPs were decorated on biodegradable nanofibers, which were harnessed for food preservation and packaging, shelf-life elongation of cheese and mitigating *Listeria monocytogenes*[21]. Besides commonly used Ag-NPs, magnetic nickel nanoparticles have also been employed after coating evenly with polyacrylic acid biofilm nanolayer, which served as an immobilisation matrix for antimicrobial peptide LL-37[81]. In another study, *Lactobacillus acidophilus* CH1 bacteriocins capped gold nanoparticles were used for targeting fungal spores (*Enterocytozoon bieneusi*), reduction[82] and alleviation in spore extrusion and, consequently, infection was observed.

Several lantibiotics also exhibit antimicrobial effects against veterinary pathogens; hence, such antimicrobials are extensively employed in livestock

and poultry. The bacteriocin's origin in traditional fermented foods and its bioengineering capacity contribute to its heightened flexibility as a potential antibiotic substitute, frequently administered in animal feed[23]. Recently, the combinatorial application of LAB bacteriocin and antibiotics for livestock and poultry has been proposed. The synergy of LAB bacteriocin with other bacteriocins or biomolecules, viz. nisin and citric acid, against *L. monocytogenes* and *S. aureus*, has also been established[19]. Compared to conventional antibiotics, resistance to bacteriocins is minimal, contributing to another reason to prefer bacteriocin over antibiotics in animal feed.

Plant growth promotion constitutes another key application of bacteriocin, which may enhance plant/crop yield by combating plant pathogens. Currently, with the ongoing challenge of climate change, enhancing crop productivity has become imperative. The phytomicrobiome includes diverse plant growth promoting bacteria/rhizobacteria that generate various bacteriocins. For instance, thuricin 17, produced by *B. thuringiensis* NEB17 and isolated from soybean nodules, has been identified as a bacteriocin that significantly contributes to boosting the photosynthetic rate[83]. Additionally, it plays a crucial role in the synthesis of certain phytohormones and modifies the root structure to facilitate improved water and nutrient uptake.

The utilization of agricultural waste for the growth of LABs and subsequent bacteriocin production offers a sustainable model of value-added transformation and control of bacterial pathogens in the composted product. The produced bacteriocin can be used reversibly in this process to preserve vegetables. In the realm of food preservation, bacteriocins have found application in diverse formats. These include their direct utilization in purified or semi-purified states, whether as part of packaging or coatings or as cell-free supernatants. Frequently, they are used in conjunction with other antimicrobial agents to combat foodborne pathogens and safeguard a variety of food products. The direct application of Fermencin SA715, derived from *L. fermentum* GA715 of goat milk origin, to the surface of bananas, not only doubled their shelf life but also ascertained the safety of fresh bananas from a microbiological standpoint[84]. For meat preservation, the semi-purified BacFL31 has been used to extend shelf life[85]. Researchers have combined bacteriocins with other antimicrobials like cinnamaldehyde and lactobionic acid for the preservation of dairy products[86]. Additionally, nisin, in conjunction with Phage endolysin PlyP100 and Caprilic acid, has been employed to target *Listeria monocytogenes* and *Salmonella*, respectively, for preserving Queso Fresco (cheese products)[87,88]. Bacteriocins have also been utilized in the form of cell-free supernatant for preserving "prato" cheese[89], and meat[90]. Freeze-dried bacteriocin producing LAB cultures have become readily accessible for use as starter cultures in food products, exemplified by the commercial product Bactoferm™ for salami[43]. Additionally, various class II bacteriocins are commercially available as food-grade fermentates containing pediocin-PA-1[61,91], Micocin®[90], which have received approval for use as biopreservative in various countries. The introduction of purified bacteriocins directly into the food diminishes their antimicrobial efficacy, as a result of the interaction between the peptides and food constituents. Nano or microencapsulation is a technique used to safeguard biopreservatives from a range of adverse conditions, and a regulated release of these agents proves highly effective in combating both microbial contamination and food spoilage. Bacteriocins, in combination with other food-grade antimicrobials, have been reported as a potential substitute for chemical additives in a range of culinary items (Table 3).

Shelf life of food can be enhanced by utilizing encapsulated bacteriocins, all while preserving their nutritional content and sensory attributes. Nonetheless, the optimal choice of encapsulation method and the most appropriate wall materials for encapsulating bacteriocins necessitate a thorough assessment of various factors, which include encapsulation efficiency, biological characteristics of the enclosed bacteriocins, release kinetics, the compatibility, and interactions between the bacteriocins and the produced microcapsules[92]. Antimicrobial peptides have been largely encapsulated by various encapsulation methods viz. liposomal entrapment[93,94] via reversed micelle method[95], thin film hydration method[96], complex coacervation[97], spray-drying[98–100] and emulsification[101], for prolonged shelf life of food or dairy products. A range of encapsulants encompassing polysaccharides like alginate[102–104], alginate-cellulose nanocrystal[105], soy/marine-lecithin[106], phosphatidylcholine[93] phosphatidylglycerol, cholesterol, porous starch/maltodextrin[107], and zein[108] have been utilized in the evaluation of suitable wall materials capable of preserving both the antimicrobial activity and structure of the bacteriocin. Consequently, no loss in organoleptic properties, quality, or nutritional value of food products was observed. Therefore, nano-encapsulated bacteriocins have been successfully employed as a protective film for dairy products like cheese[21,109], meat, and ready-to-eat product packaging[105] and seem to be a sustainable approach for food preservation.

Bioengineering: a go to technique for development of tailored bacteriocins

The ribosomal origin, tolerance, and flexibility of bacteriocins in bioengineering render them preferable as antibacterial agents compared to classical antibiotics[110]. LAB bacteriocins, derived from ethnic fermented foods, are particularly compelling as ideal candidates in food systems. In addition to probiotic source, the successful application of bacteriocin in food systems depends on crucial factors such as heat stability, solubility, and its distribution within the food system[111]. With the growing interest in bacteriocins, numerous studies have focused on the bioengineering of lantibiotics to produce pharmacologically more potent derivatives, specifically targeting gram-positive pathogens. The leader peptide emerges as a crucial target for modification as acted upon by post-translational modification enzymes, making it the primary focus for developing bacteriocins with desirable properties. Previously, incorporation of hydroxy acids into lacticin 481 and nukacin ISK-1 in *E. coli* has been undertaken, enabling the connection of the leader peptide through an ester linkage that is subsequently cleaved via hydrolysis[112]. Nisin, widely investigated among all bacteriocins following its commercial approval for use in food systems, is particularly sought after for modification. Recently, with an aim to pharamacologically improve the nisin A, non-canonical amino acids (ncAAs) have been incorporated genetically into Nisin A[113] which led to formation of a novel ring A thioether bond for one of the mutants leading to overall property enhancement of bacteriocin. In another recent research, lapidated variant of nisin was developed by insertion of ncAAs in nisin taking *L.lactis* as a host for escalated efficiency and concentration. Specifically, ethionine was incorporated which improved the overall bioactivity and azidohomoalanine (Aha) incorporation alongwith click chemistry application led to the development of lapidated variants which were found to be effective against various gram-positive pathogens[114]. In continuation with this work, a variant of nesin, cesin R15G has been developed by the same research team. This variant maintained its antimicrobial activity while successfully overcoming trypsin degradation. Unlike other nisin analogs, this variant, lacking two terminal macrocycles, demonstrated broad antimicrobial activity when expressed heterologously in *L.lactis*[115].

Lab bacteriocin: favoourable characteristics and challenges concerninng food industry

A bacteriocin is rendered ideal for application in a food system if it exhibits extant properties/traits that ensure its functional viability in food and non-toxicity to the human body. They are produced in nature by a diverse array of prokaryotic and eukaryotic organisms, yet very few can be applied to the food industry. In general, it has been observed that some bacteriocins exert some undesirable properties that limit their applicability in food and health sectors. Some bacteriocins are toxic to eukaryotic cells and induce haemolysis of RBCs, show a limited pH range of action, narrow spectrum of antimicrobial activity, reduced antimicrobial activity in complex food matrices and serum, and are highly sensitive to proteases. Moreover, the downstream processing methodology employed for post-fermentation antimicrobial peptide purification plays a key role; thus, bacteriocin shall pass convenient purification processes such that its large-scale purification at the commercial level becomes feasible.

Table 3 | Combinatorial effect of bacteriocin with other food grade antimicrobials for the preservation of food products

LAB Bacteriocin	Producer organism	Antimicrobial (coating/composition/ biopreservants/Nanobioconjugate	Technique used	Food product	Application targeting food safety	References
Nisin	*Lactococcus lactis*	Nisin-loaded amaranth protein isolate:pullulan (API:PUL) nanofibers, scope to use as packing material	Electrospinning	fresh cheese and apple juice	Improved antibacterial activity and prolonged release.	89
Nisin	*Lactococcus lactis*	Nisin-lysozyme encapsulated in Soybean phosphatidylcholie nanoliposomes	Thin film hydration method	Skim milk	Antibacterial effect (*Listeria monocytogens*, but not *Salmonella* Enteritidis)	90
Nisin	*Lactococcus lactis*	Nisin-loaded zein microcapsules	Microfliuidic technique	Queso fresco cheese	Anti-listerial activity, (2 log CFU/gm reduction)	91
Nisin	*Lactococcus lactis*	Isolates of whey protein with garlic oil/nisin/ oregano oil/natamycin, films fused with packaging film /low-density polyethylene film	__	Kasar cheese	Decreased *S. aureus* counts by 2.15 log CFU and *E. coli* O157:H7 by 1.48 log CFU	132
Nisin	*Lactococcus lactis*	Milk phospholipids-based nanostructures functionalized with rhamnolipids and nisin	Ultrasonication-assisted self-assembly method	Cheese	Prevention of food borne pathogens	96
Nisin	*Lactococcus lactis*	Cinnamon nanoessential oil capsules	__	Beef	Quality and shelf life preservation	108
Pediocin	*Pediococcus pentosaceus*	Pediocin decorated silver nanoparticles (Pd-SNPs) wrapped in biodegradable nanofibers (Packaging material)	Electrospinning	Cheese	Antilisterial effect	21
Bacteriocin	*Lactobacillus pentosus S6 (KU92122), Lactobacillus spicheri G2 (JX481912) & Lactobacillus crustorum F11 (KT865221)*	Bacteriocin synthesized AgNPs coated cellulose paper	Silver nitrate based chemical method	Tomato	Shelf-life extension	133
Nisin	*Lactococcus lactis*	Polyvinyl Alcohol / Polyacrylate Sodium Nanofiber Containing Nisin-Loaded Nanoparticles	Sonication, Electrospinning	*Strawberry*	Prolonged Shelf life	134
Nisin	*Lactococcus lactis*	Microcapsule of collagen/pectin carrying Nisin and avocado peel extract	Complex coacervation	Ground beef	Prolonged Shelf life	135
Nisin A	*Lactococcus lactis*	Nisin A loaded beads from alginate/hi-maize resistant starch	Microencapsulation	Cheddar cheese	Gradual release of bacteriocin facilitatedProlonged Shelf life	136
Divergicin M35	*Carnobacterium divergens*	Divergicin M35 loaded chitosan packaging film		Ready-to-eat, minimally processed products	Antilisterial effect	137
Nisin	*Lactococcus lactis*	Nanofibers with polyethylene packs	Electrospinning	Rainbow trout fillets	Off-odor prevention and reduction in microbial growth	97

Desirable attributes

An ideal bacteriocin for food preservation should possess several key characteristics: it should originate from a GRAS microbe, exhibit high antimicrobial activity, maintain its effectiveness in complex food matrices, and have a prolonged half-life. Additionally, it should have minimal binding to serum components, be non-toxic to mammalian systems, resilient to proteases and environmental conditions, and amenable to low-cost production and purification methods.

In general, amino-acid chain length, the net charge on the functional peptide, the presence of hydrophobic residues, and the amphiphilicity of the peptide are crucial points for the potent antibacterial activity of bacteriocins. Further, the application in food systems requires thermal stability. Their eventual medical application (if desired) would require serum stability also. Since these antimicrobials are sensitive to proteases, the ideal bacteriocin must be stable against proteases present in food systems. Most food-grade bacteriocins are produced extracellularly and secreted in the medium. Moreover, the downstream processing methodology employed for post-fermentation antimicrobial peptide purification plays a key role; thus, bacteriocin shall pass convenient purification processes such that its large-scale purification at the commercial level becomes feasible. The candidate bacteriocin should also pass the toxicity barrier for successful functioning in the gut. Though some scant studies have studied the effect of bacteriocins on the gut microbiome, it is expected that bacteriocins should not modulate the microbiome in an undesirable manner. A separate discussion will focus on the influence of bacteriocins on the regulation of the gut microbiome.

Another vital aspect that needs to be considered before utilizing antimicrobial peptides in food, human, or animal health is assessing the development of resistance to bacteriocins over extended exposure to a target bacterial strain. It is also a prerequisite to assess the resistance development towards bacteriocin use for a long duration in target cells before its application in food or medical purposes. Lastly, co-resistance should be addressed before employing a bacteriocin towards a specific pathogen to ascertain whether the pathogen is susceptible to a bacteriocin from the same class it produces[19].

It is imperative to expect that a single bacteriocin will fulfil all the desired criteria. Thus, researchers have paid attention to modifying the bacteriocin sequences to engineer them towards desirable properties or use them with other materials like nano forms to generate nano-bio hybrids with desirable properties[116]. Since the food safety testing of modified peptides (including modified recombinant variants) needs evaluations and approvals from authorities, the future miletone should be to emphasize on generating nano-bio hybrids with proper toxicity and safety evaluations.

Potential bottlenecks

The distinctive qualities of bacteriocins, viz. extracellular production, potent activity against target organisms, heat, salt, and pH tolerance, position them as a viable choice among available synthetic or traditional antimicrobial agents for both food preservation and healthcare applications. Although bacteriocins have demonstrated effectiveness, there exist significant limitations that might potentially hinder their use as antimicrobial agents. Efforts are being made to develop strategies to address these limitations. A deeper understanding of the potential bottlenecks can motivate researchers to make bacteriocin more effective for food applications.

Limited antibacterial spectrum

Bacteriocins exhibit either a narrow or broad spectrum of antibacterial activity. Broad-spectrum bacteriocins are deemed effective for biopreservation of food due to their presumed safety and extensive antibacterial properties. However, their application as therapeutic agents raise concerns about potentially upsetting the balance among microbiota phyla, leading to a rise in proteobacterial population and alleviation in bacteroidetes and firmicutes, akin to the effects of broad-spectrum antibiotics[11]. Conversely, the narrow spectrum of certain bacteriocins is advantageous for maintaining microbiota balance. In this scenario, the identification of the specific pathogenic bacterium is crucial before deploying such bacteriocins for therapeutic purposes. Additionally, the specificity of narrow spectrum bacteriocins in targeting specific bacteria helps alleviate selective pressure on non-targeted microbes[117].

In vivo efficacy

In a nutshell, pharmacokinetic properties and administration route are two key determinants of bacteriocin's in vivo efficacy[118]. The enzymatic breakdown of bacteriocins by proteases within the gastrointestinal tract renders them suitable for safe intake by humans and animals. Nevertheless, the proneness of bacteriocins to proteolytic breakdown in the gastrointestinal tract is a significant obstacle to their oral administration. This vulnerability to in vivo proteases is expected to result in a shorter half-life compared to their antibiotic counterparts[119]. Low plasma and metabolic stability constitute another drawback of bacteriocins, which may limit therapeutic/medical applications. However, such issues can be addressed by developing bio-engineered variants of natural bacteriocins. Studies investigating peptide modification of bacteriocins have provided valuable insights into addressing this problem[114,115]. Previous research has explored the potential of modifying bacteriocins to eliminate recognition sites for proteases. By examining the structural information obtained from these studies, it is possible to develop strategies to effectively address this issue. Moreover, to combat proteolytic degradation, parenteral administration provides a viable option[118], especially in the case of systemic infections as far as medical applications are concerned[120]. In addition to this, encapsulation techniques can be applied for both food bio-preservation and healthcare applications.

Large scale production, purification, and high cost

Given the high demand for bacteriocins in both the medical and food sectors, achieving cost-effective mass-scale production is of paramount importance. The complex nature of bacteriocins poses challenges in large-scale production and purification, leading to significantly high production costs, rendering their synthesis impractical for widespread use[121]. In addition to these, reports have indicated drawbacks such as lack of sufficient explanations of mechanism of action and alleviation of efficacy in intricate multifaceted environments[73]. Primarily, insufficient recovery upon purification leads to low yield[122], hence, it is essential to explore methodologies that specifically aim for high yields.

Lastly, the absence of cytotoxic assessments is another significant hurdle in the advancement and application of bacteriocins for therapeutical purposes, particularly as antimicrobial therapeutic agents[19]. This lack of evaluation hinders their potential utilization in clinical settings. Some studies have been conducted to develop cost effective media, protein engineering and charge based facile purification method to address this issue.

Roadmap beyond conventional antimicrobials

Bacteriocins from fermented foods have gained traction in terms of their promise to improve the food shelf life, mitigate post-harvest losses in agricultural produce and offer a plethora of other applications in the food industry. There is a dearth of methodologies targeting disease management, specifically gut health enhancement through functional food production involving bacteriocins and their producer organism. In the present era of increasing episodes of AMR, dissemination of AMR can be alleviated by employing such small peptides as smarter antimicrobials, which can serve as potent alternatives to classical antibiotics owing to their antimicrobial nature. Encapsulation of probiotic bacteria has been explored earlier; following its lead, the development of functional food may be achieved by designing a nano-in-microsystem for successful delivery of LAB in conjunction with micronutrients, bioactives, and metal nanoparticles decorated with bacteriocins for improved gut health. Implementing this integrated approach would optimize the simultaneous delivery of all these components, establishing a versatile vehicle for advancing gut health. Furthermore, diverse LAB bacteriocins from fermented foods can be analysed in vitro to comprehend microbiome dynamics in the gut. To maintain the physicochemical attributes of vegetables and mitigate post-harvest losses, transparent packaging films containing tailored bacteriocin-nanoparticle complexes may be employed to fabricate nanocomposite antimicrobial films

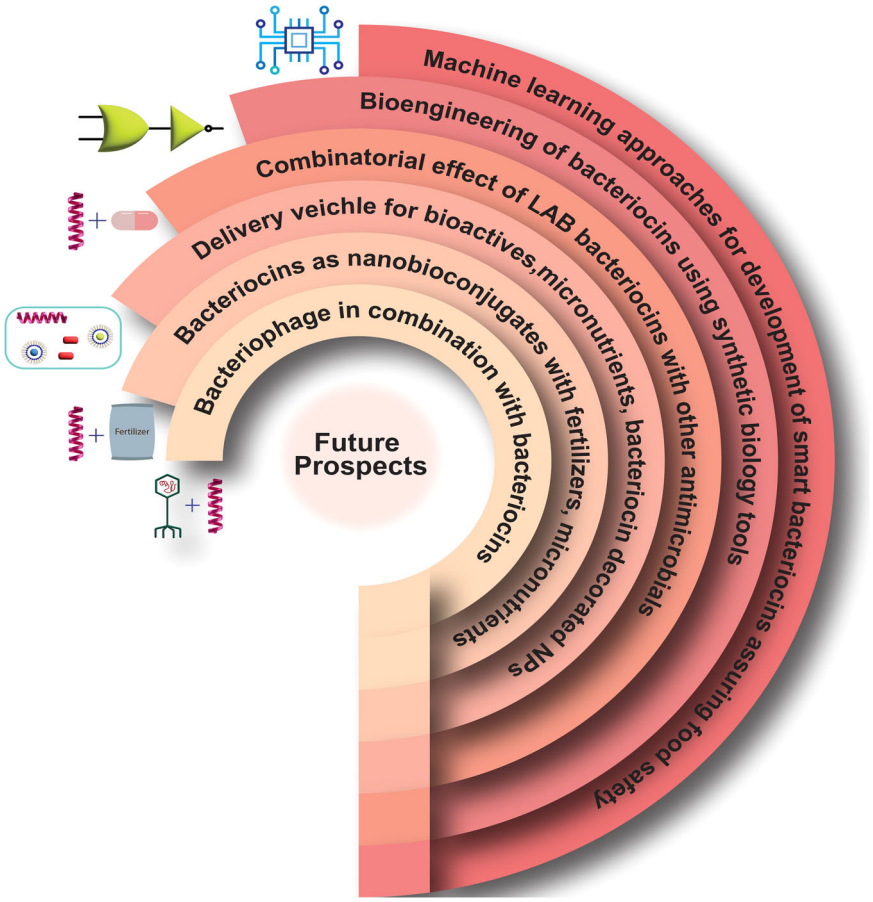


Fig. 5 | Prospective approaches for the evolution of bacteriocin into an ideal biopreservative for application in the food sector. Future endeavours in the realm of bacteriocins can explore several advanced approaches to overcome challenges associated with their large-scale production and contribute to sustainable development goals. These strategies encompass bioengineering, application of machine learning algorithms, investigation of the synergistic effect of native bacteriocins with potent antimicrobial agents, and phage endolysins addressing a broader spectrum of bacterial infections. Additionally, the development of nanobiotics and their sophisticated delivery vehicles can revolutionise targeted transportation across multiple sectors for imparting nutritional and therapeutic benefits.

employing low-density polyethylene. An additional intriguing facet of bacteriocins is their potential application in horticulture as nanocomposites coupled with fertilizers, alleviating plant pathogens, enhancing overall crop health and yield. The utilization of bacteriocins, coupled with fertilizers containing macro and micronutrients in the form of nanocomposites, can be sprayed onto crops. This application allows for controlled release, specifically targeting pathogens and enhancing the health of the crops. Targeting antimicrobial activity in food products, phage endolysins have also been explored, however, phage or phage cocktails, engineered endolysins with broad spectrum antimicrobial activity can also be employed in combination with FDA-approved bacteriocin to address temperature resilience, solubility, target specificity, and cost-effectiveness challenges on a larger scale.

Combating the burgeoning demand for bacteriocins in the food and agriculture industry requires environmentally friendly and cost-effective strategies. Utilizing dairy industry waste emerges as a promising and viable option. Omics-based technologies can be leveraged to focus on LAB bacteria, using *in-silico* genome mining to discover novel antimicrobial peptides. This approach can efficiently expand the existing pool, reducing the need for time-consuming, labor-intensive processes.

Moreover, machine learning algorithms may be employed for transforming bacteriocins into an optimal biopreservative suitable for use in the food industry. Figure 5 depicts potential future avenues for the advancement of highly effective bacteriocins in the context of the food sector, considering sustainable developmental goals. Considerable efforts have been invested in enhancing the overall characteristics of bacteriocins to address the challenges related to food preservation. Bioengineering and synthetic biology have great potential and are practical platforms. Future research should investigate a large inventory of LAB bacteriocins for bioengineering, especially regarding food safety, mass scale bacteriocin production which is believed to foster the development of smart bio-based material.

Supplementary information

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Author contributions

All authors contributed to the study conception and design. V.B., B.D. collectively wrote the manuscript and made the tables and figures. A.H., V.K., N.K.N. revised the manuscript.

Competing interests

The authors declare no competing interests.

Additional information

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