BACTERIOCIN-PRODUCING PROBIOTICS: APPLICATION IN THERAPEUTICS

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Abstract

The use of traditional dairy products as a source of beneficial microorganisms has gained significant attention due to their rich microbial diversity and potential health benefits. Among these microorganisms, lactic acid bacteria (LAB) are particularly notable for their probiotic properties, including the production of bacteriocins antimicrobial peptides that inhibit the growth of pathogenic bacteria. Bacteriocins offer a natural and effective means of food preservation and have therapeutic potential in combating antibiotic-resistant pathogens, making them a subject of growing interest in both food and pharmaceutical industries. Traditional dairy products, often fermented through natural processes, serve as a reservoir for diverse strains of LAB capable of producing unique bacteriocins. These products, such as yogurt, cheese, and fermented milk, provide an ideal environment for the growth of LAB, fostering the development of unique microbial communities. The isolation and characterization of these probiotic LAB strains, particularly those producing bacteriocins, are crucial for understanding their role in food safety, human health, and their application in developing novel functional foods. This review is based on the exploration of bacteriocin-producing LAB from traditional dairy products offers valuable insights into their diversity, functionality, and potential applications. By examining recent findings, this paper will highlight the potential of these strains in enhancing food safety and developing new therapeutics, thereby contributing to the broader understanding of their role in human health and nutrition.

Keywords: Lactic acid bacteria, Traditional dairy product, bacteriocin, antimicrobial activity, Prebiotic, parabiotic, neurobiotics.

1. Introduction:

Lactic acid bacteria (LAB) are a diverse group of Gram-positive bacteria known for their ability to ferment carbohydrates into lactic acid, playing a crucial role in the production of a wide variety of fermented foods. Beyond their fermentative capabilities, LAB have gained significant attention for their production of bacteriocins—antimicrobial peptides that inhibit the growth of pathogenic and spoilage bacteria. LAB bacteriocins are considered safe and effective due to their natural origin, specificity towards target organisms, and minimal impact on human cells, making them highly desirable in food preservation and as potential therapeutic agents. LAB bacteriocins are produced by various strains of LAB, such as Lactobacillus, Lactococcus, Pediococcus, and Enterococcus. These bacteriocins are classified into different classes based on their structure, size, and mode of action.

Different countries have established the minimum number of viable cells needed in a probiotic product to be useful. In Australia, a minimum viable count of 10 organisms per gram must be present in fermented milk products at the end of shelf life. However, according to (Krasaekoopt et al. 2003), there are no specifications for the amount of probiotics that should be present in Australian fermented products.

The ability of LAB to produce bacteriocins in situ during fermentation adds an extra layer of protection to food products, enhancing their safety and extending shelf life. Given the increasing demand for natural preservatives and alternatives to antibiotics, LAB bacteriocins represent a promising solution. The review article focuses on the exploration of bacteriocin-producing LAB from traditional dairy products offers valuable insights into their diversity, functionality, and potential applications.

2. Biosynthesis of LAB Bacteriocins

Bacteriocins are biologically active, ribosomally synthesized peptides, primarily produced by Lactobacillus spp., with significant antimicrobial properties. Isolated from sources like cottage cheese and curd, these heat-stable, low-molecular-weight peptides exhibit diverse structures and functions, interacting with target cell membranes to disrupt integrity. Their broad or narrow-spectrum activity makes them valuable in food preservation, medicine, and agriculture, as they selectively kill specific bacterial strains (Kjos et al., 2010). Bacteriocin biosynthesis involves gene clusters encoding structural, immunity, and accessory genes, leading to precursor peptide synthesis, post-translational modifications, secretion via ABC transporters in Pediococcus acidilactici, proteolytic activation, and immunity mechanisms to protect producing bacteria (Guder et al., 2000; Fimland et al., 2005). These peptides, exemplified by nisin and pediocin, hold potential as innovative antimicrobial agents (Lubelski et al., 2008).

3. Classification of LAB Bacteriocin

Most LAB bacteriocins are small cationic peptides (less than 10 kDa), heat stable, amphiphiles and membrane penetrating peptides. They can be divided into three main classes (Fig. no.1), their classification has been constantly revised during the last decade due to the extensive research carried out.

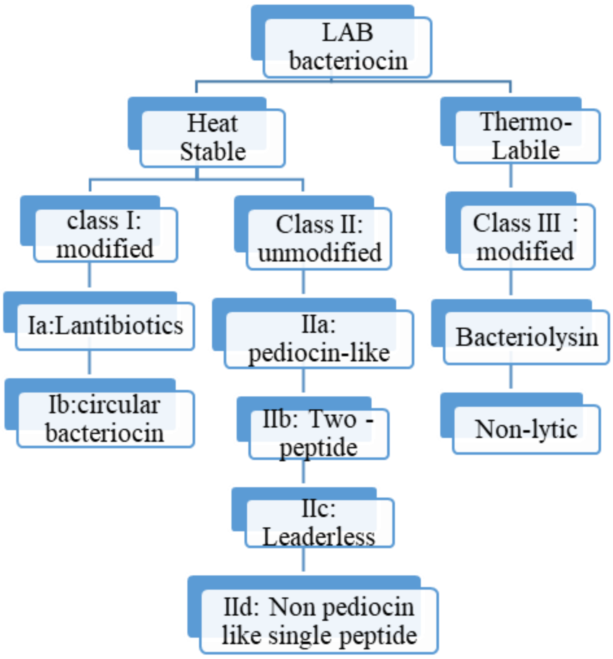

Figure 1. Diagrammatic representation of Bacteriocin classification

Bacteriocins are classified into three main classes based on their structure and properties. Class I (lantibiotics) are small, heat-stable peptides containing unique amino acids like lanthionine, undergoing extensive post-translational modifications. They are further divided into Type A, which forms membrane pores (e.g., nisin), and Type B, which interferes with enzymatic reactions (Cotter *et al.,* 2005). Class II (non-lantibiotics) are small, heat-stable peptides that do not undergo extensive modifications and include subclasses like IIa (e.g., pediocin, targeting *Listeria monocytogenes*) and IIb (bicomponent bacteriocins requiring two peptides for activity). Class III comprises larger, heat-labile proteins with bacteriolytic activity, such as helveticin I, but remains less studied. Among these, Class I and II are the most researched for their broad-spectrum antimicrobial potential and stability under diverse conditions (Nissen-Meyer *et. al.,* 2009).

Table 1. The most common bacteriocins produced by LAB are summarized in the table

Bacteriocin	**Bacteriocin Producing Agent**
Lactacin F	L. *johnsonii spp.*
Lactocin 705	L. *casei spp.*
Lactoccin G	L. *lactis spp.*
Lactococcin MN	*Lactococcus lactis var cremoris*
Nisin	*Lactococcus lactis spp.*
Leucocin H	*Leuconostoc spp.*
Plantaricin EF, Plantaricin W, Plantaeicin JK, Plantaricin S	L. *plantarum spp.*

(Zacharof *et al*., 2012)

4. Bacteriocins as Versatile Biotics: Understanding Their Prebiotic, Postbiotic, Parabiotic, and Neurobiotic Applications

4.1. Probiotics

Probiotics are live microorganisms that provide health benefits to the host when administered in adequate amounts. The production of bacteriocins by probiotic strains contributes to their ability to maintain a healthy gut microbiome, inhibit the growth of pathogens, and modulate the host's immune response. For example, *Lactobacillus acidophilus* and *Lactobacillus plantarum* produce bacteriocins such as acidophilin and plantaricin, which have been shown to effectively inhibit pathogenic bacteria like *Listeria monocytogenes* and Salmonella enterica (Cleveland *et al*., 2001). Studies have demonstrated that *Lactobacillus reuteri* produces reuterin, a bacteriocin that inhibits both Gram-positive and Gram-negative bacteria, thereby contributing to a healthier gut environment (Joerger, 2003). The immune-modulating effect has been observed in several studies, where bacteriocins like nisin from *Lactococcus lactis* stimulated the production of cytokines that regulate immune function (Dobson *et al*., 2012). Probiotics such as *Lactobacillus rhamnosus* GG produce bacteriocins that can disrupt biofilms, reducing the persistence and virulence of pathogens (Amenu and Bacha, 2024). Bacteriocins enhance the survival and stability of probiotics in the gastrointestinal tract. By inhibiting competitors and pathogens, bacteriocins create a favourable niche for the probiotic strain, improving its viability and prolonging its beneficial effects in the gut (Gonzalez *et al.,* 2022).

4.2. Prebiotics

Prebiotics are non-digestible food ingredients, usually fibers or carbohydrates that promote the growth and activity of beneficial microorganisms in the gut. While bacteriocins themselves are not traditionally classified as prebiotics because they are not food for beneficial bacteria, they can indirectly support the growth and activity of probiotics by modulating the microbial environment in a manner similar to prebiotics. For instance, bacteriocins produced by Lactobacillus and Bifidobacterium species can suppress the growth of harmful bacteria, creating a more favourable environment for the growth of beneficial microbes (And & Hoover, 2001). This selective inhibition promotes a healthier balance of the gut microbiota, which is a primary goal of prebiotic compounds. By reducing competition from harmful bacteria, bacteriocins can create conditions that enhance the growth and activity of probiotic strains. This can lead to increased production of beneficial metabolites, such as short-chain fatty acids (SCFAs), which are known to support gut health. Bacteriocins can help manage gut dysbiosis (an imbalance in the gut microbiota) by selectively targeting harmful bacteria while preserving beneficial ones. This property can lead to improved microbial diversity and health, which is a critical goal of prebiotics (Dobson *et al*., 2012).

4.3. Parabiotics

Bacteriocins, which are antimicrobial peptides produced by certain bacteria, can serve as effective parabiotic (or postbiotics). Parabiotic refer to non-viable microbial cells or their components that confer health benefits to the host. Even when the bacteria that produce them are no longer alive, bacteriocins can still exhibit beneficial effects, making them a valuable component of parabiotic formulations. As components of parabiotic, bacteriocins can help inhibit the growth of pathogenic bacteria in the gut, contributing to the maintenance of a healthy gut microbiota. For example, bacteriocins produced by *Lactobacillus* and *Bifidobacterium* species have been shown to reduce the levels of harmful bacteria, such as *Clostridium difficile* and *Salmonella* (Zhang *et al.*, 2018). Parabiotic containing bacteriocins can help strengthen the gut barrier function. By maintaining a balanced microbial environment, bacteriocins reduce gut permeability ("leaky gut") and prevent the translocation of harmful bacteria and toxins into the bloodstream, which can trigger systemic inflammation (Nataraj *et al.*, 2020). Bacteriocins as parabiotics also have a longer shelf life and greater stability, making them easier to use in various food and supplement formulations (Salminen *et al.*, 2021).

4.4. Neurobiotics

The concept of bacteriocins as neurobiotics is still in its early stages, but emerging research suggests that bacteriocins—antimicrobial peptides produced by certain bacteria—may influence the gut-brain axis and, consequently, brain health. Bacteriocins can help maintain a healthy gut microbiota by inhibiting pathogenic bacteria that may negatively affect the gut-brain axis. This microbial balance is crucial for the production of neuroactive compounds like serotonin, dopamine, and GABA, which are essential for brain function and mood regulation. Studies suggest that bacteriocins, such as those produced by Lactobacillus species, help preserve beneficial bacteria that contribute to the synthesis of these neurotransmitters, thereby indirectly supporting mental health and cognitive function (Avsar *et al.*, 2020). Neuroinflammation is increasingly recognized as a key factor in various neurological and psychiatric disorders. By inhibiting harmful bacteria that can trigger systemic inflammation, bacteriocins may reduce the risk of neuroinflammation. For instance, *Lactobacillus plantarum* produces bacteriocins like plantaricin, which can help suppress gut pathogens and thereby reduce the inflammation that may otherwise affect the brain. Bacteriocins may play a role in enhancing gut barrier function by preventing the overgrowth of harmful bacteria that contribute to "leaky gut." When the gut barrier is compromised, toxins and inflammatory molecules can enter the bloodstream and potentially reach the brain, contributing to neurological disorders. Bacteriocins, by promoting a healthy gut microbiota, help maintain the integrity of the gut barrier, indirectly protecting brain health (Hemarajata and Versalovic, 2013). Certain bacteriocins may impact mood and behaviour by reducing the levels of lipopolysaccharides (LPS), endotoxins produced by Gram-negative bacteria that can promote anxiety and depression-like behaviours. By targeting bacteria that produce LPS, bacteriocins may contribute to a decrease in these behaviours (Kim *et al.*, 2024).

5. Applications of Bacteriocin

Bacteriocins, antimicrobial peptides produced by lactic acid bacteria (LAB), have numerous pharmaceutical applications due to their targeted action against pathogens, minimal impact on beneficial microbiota, and therapeutic potential. They serve as alternatives to conventional antibiotics, effectively combating resistant strains like MRSA and VRE. Bacteriocins aid in managing gastrointestinal disorders (e.g., IBD) by targeting pathogens while preserving gut flora and show promise in cancer therapy, inducing apoptosis in cancer cells through mechanisms like ROS accumulation and mitochondrial disruption (Kaur & Kaur, 2015). They also modulate the immune system, stimulate cytokine production, and are effective in treating skin infections and wounds, such as nisin-based formulations for *Staphylococcus aureus*. Additionally, bacteriocins can disrupt biofilms, making them valuable in preventing infections on medical devices and dental materials. They are also explored as neurobiotics, promoting brain health by modulating the gut-brain axis, offering potential benefits for neurodegenerative disorders.

Conclusion

The exploration of bacteriocins from lactic acid bacteria (LAB) isolated from traditional dairy products reveals their significant potential as natural antimicrobial agents with broad applications in food preservation and human health. Traditional dairy products, rich in diverse and unique LAB strains, offer a promising source of bacteriocins that can enhance food safety by inhibiting pathogenic microorganisms and extending shelf life. The detailed understanding of their genetic makeup, physicochemical properties, and antimicrobial spectrum underscores the versatility of bacteriocins in combating a wide range of foodborne pathogens. Moreover, the probiotic nature of these LAB strains adds an additional layer of health benefits, including gut microbiota modulation and immune system support, making them attractive candidates for functional foods and therapeutic applications. However, challenges such as optimizing production, ensuring safety, and meeting regulatory requirements must be addressed to fully harness the potential of bacteriocins. In conclusion, bacteriocins from LAB in traditional dairy products represent a powerful tool in the ongoing quest for natural and effective antimicrobial agents. Future research should focus on overcoming current challenges and expanding the application of these natural compounds in both food and medical industries, thereby contributing to improved food security and public health.

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