Exploring the versatility of a bio-preservative bacteriocin: Application in health and allied sciences

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***Abstract* :** Global food trade has required the centralization of production and storehouse systems with potential problems for food security and safety as concerns mount about increases in population growth. Consumer and safety pressure means some traditional preservation techniques like drying, salting)are being used in conjunction with those using chemicals compounds (bactericides) or ones base on pasteurization. Bacteriocins, ribosomally synthesized antimicrobial peptides mainly produced by lactic acid bacteria (LAB), have become an attractive food preservative. They exert their function by forming pores on bacterial membranes, inhibiting peptidoglycan layers formation in the cell wall, interfering with essential enzymes and causing apoptosis-like processes. Lantibiotics are classified in three major classes which includes Class I (Lantibiotics), II (Non-Lantibiotics while subdivided as above) and III(large heat labile proteins). Food preservative bacteriocins are chosen according to their wide spectrum of activity, safe status, stability and compatibility with food matrices as well as feasibility for production. This may enable applications with living organisms, such as probiotics which improve shelf life and safety in food products including meat, seafood, dairy as well packaging films to prevent spoilage by pathogenic bacteria. They can provide an environmentally friendly alternative to the chemical preservatives and also respond to consumer trends of safer, more natural products.

Keywords : preservatives, shelf-life, food spoilage, bacteriocins, lab

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INTRODUCTION

The distribution of food items from centralised manufacturing firms and the large-scale storage of food products have been mandated by the globalisation of the food trade magnitude. Concerns about food security and safety have spread around the world along with population growth. Because fresh foods and minimally processed foods block other microorganisms and food-borne diseases, they pose a new threat to food security and safety. Processes used to preserve food often involve drying, salting, heating, and cold storage.
The use of chemical compounds, pasteurisation, and canning are some contemporary methods for extending the shelf life of food products. Certain traditional preservation methods have been disapproved in accordance with consumer desires, stringent food approval regulations, and food safety requirements. involves seasoning the meal with salt and a few preservatives such sorbic, benzoic, and acetic acids. Customers who used these preservation methods experienced some adverse reactions. Additionally, nitrites, or nitrosamines, are formed, which might cause cancer. [1] The necessity of selecting, enhancing, and producing beneficial microbial products has increased within the food industry due to the growing need for biopreservation strategies. sectors. Bacteriocins have gained interest as a potential new generation of food preservatives in the hunt for bio preservatives that can replace chemical preservatives. [2] Groups of ribosomally produced antimicrobial peptides known as bacteriocins have the capacity to either suppress or kill closely related or unrelated bacterial strains without endangering the organism itself.[3]

The first bacteriocin to receive FDA approval for use in the preservation of pasteurised processed cheese spread is nisin.[4] Because lactic acid bacteria (LAB) produce nisin, pediocin, and other bacteriocins that are beneficial to human health, food production, and the replacement of chemical preservatives whose safety is constantly under question, these preservatives have garnered a lot of attention.[5] The bacteriocins derived from Gram-positive bacteria have the following properties: broad spectrum activity against both Gram-positive and Gram-negative organisms as well as certain fungi; restricted spectrum action, active against related types of organisms.[6]

CLASSIFICATION OF BACTERIOCIN

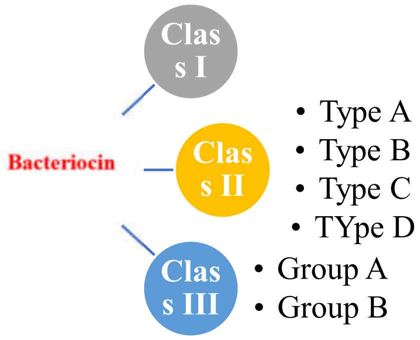

Fig.1: Classification of bacteriocin

Class I – Lantibiotics

- Peptides are a kind of bacteriocins that are post-translationally modified membrane active peptides that contain smaller than 28 amino acids small peptide (<5 kilodalton) ; they can either be linear and, in this case, membrane disrupting, or the peptides are globular are cellular enzymes.
- Classification of Bacteriocin Inlist In Fig.1.
- **MOA-** lantibiotics Primarily target vulnerable bacteria's cell membranes. They frequently damage membrane integrity by forming pores or channels, which lead to leaking of cellular material and finally cell death. **Examples of Class I-** Nisin , Mersacidin & Lactosin.[7]

Class II – Non – Lantibiotics

- Bacteriocins are circular peptides with heat resistance. Class II bacteriocins have 30-60 A.A-amino acids (<10kDa) and are unaltered. **Mode of Action-** In general, they have an amphiphilic helical shape. This allows them to enter into the target cell's membrane. This leads to depolarization and death. Class II bacteriocin can be organised into 3 subclasses:

1. **Type A**: High antimicrobial activity.There have 37-48 amino acid residue present in the molecular structure of these bacteriocin.Eg. Pediocin, sakicin, levcocin, produced by leuconostoc gelidium.
2. **Type B:** Non-Lantibiotic , two peptide bacteriocin. It consist of two different peptide that act synergistically to exert antimicrobial activity. Eg. Lactococcin G and Lactococcin M produced by Lactococcus lactins.
3. **Type C:** Circular bacteriocin. Which is formed by head to tail cyclization of the peptide chain. Eg. Enterosin AS 48 produced by intercoccus faecalis. Circular a produced by clostridium beljerincki. Enteriocin and neuterin
4. **Type D:** Linear bacteriocin. That lack the unusual amino acid found in lantibiotics. Eg. Plantaricin C Produced by lactobacillus plantarum. Enteriocin L50 produced by enterococcus faecium.[8]

Class III- Large heat lable Proteins. These are group of antimicrobial peptide Produced by bacteria. unlike class I and class II bacteriocin. which are small peptide. class III bacteriocin are larger Protein with molecular weight typically greater 30 kDa. These bacteriocin normally produced by gram- negative bacteria. class III can be further subdivided into two distinct geoups.

GROUP A bacteriocins are bacteriolytic enzymes that kill susceptible strains by lysing their cell walls, such as Enterolysin A. **Group B** bacteriocins are non-lytic proteins such as caseicin.[9]

MODE OF ACTION

A) Pore Formation in the Target Cell Membranes

Most commonly, the mechanism used is through formation of pores in the cell membrane of bacteria. For this purpose there are well known examples as nisin (a lantibiotic) and pediocin that bind to molecular target on surface of the receptor bacteria [10]. When these bacteriocins bind, they insert themselves in the membrane and aggregate to form pores/channels. These pores cause destabilization of the membrane and in turn:

• Leakage of Intracellular Contents: Molecules such as ATP, ions and metabolites leak out form the cell.

• Depolarisation: Depolariation is the condition in which cells looses membrane potential, affecting generation of energy and transportation of nourishment.

• Cell Death: Over time, these disruptions cause complete cell death.[10] Diagramatic MOA of Pore Formation in Target Cell Membranes Show in Fig.2.

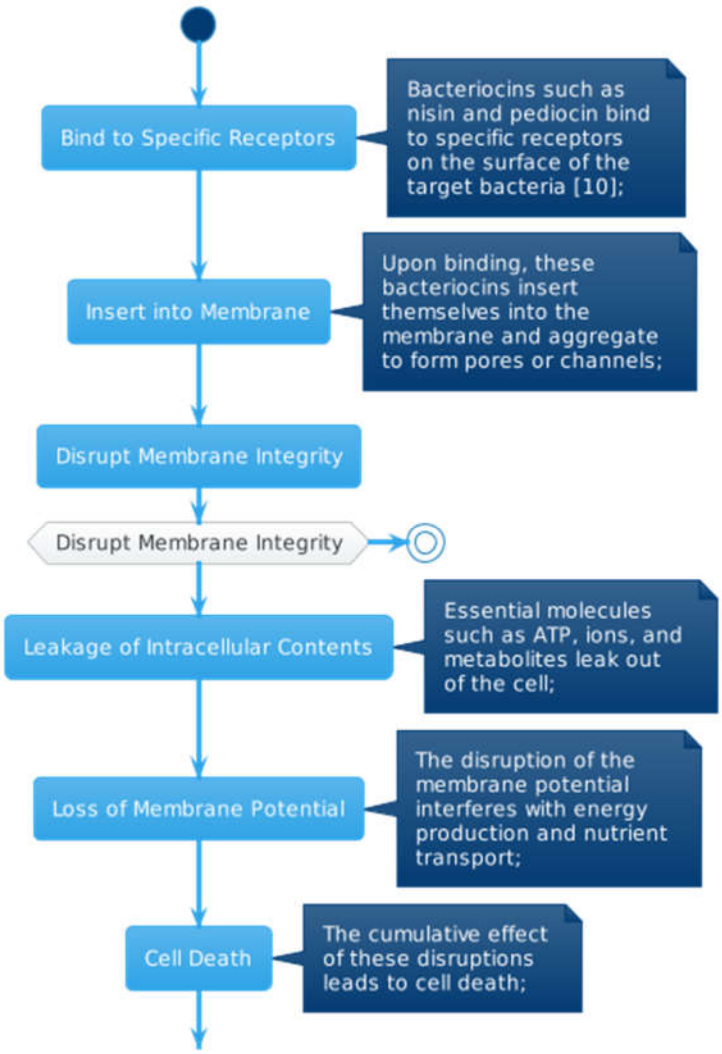

Fig.2: Pore Formation in Target Cell Membranes

B) Inhibition of Cell Wall Synthesis

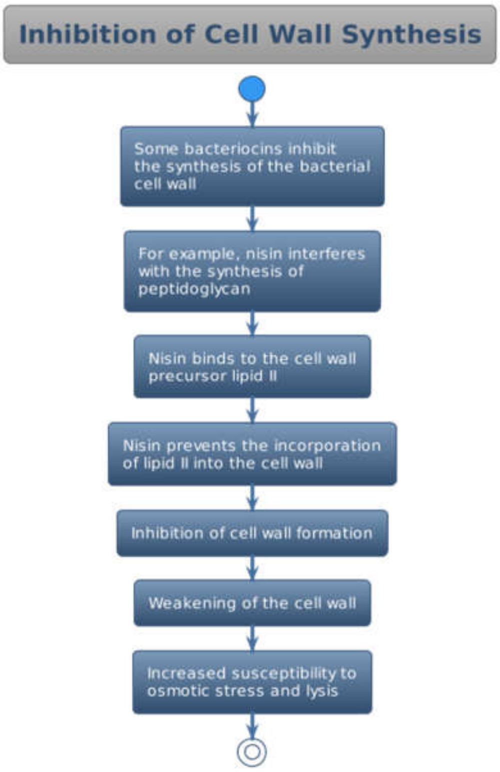

Fig.3: Blocking the Formation of Cell Walls

Certain bacteriocins such as lantibiotics and peptides have been elucidated with specific action inhibiting the synthesis of bacterial cell wall formation, which is an essential component for growth and establishment of bacteria. Thus, nisin works by interrupting the synthesis of peptidoglycan in Gram-positive bacteria which is a vital part of their cell wall. In ordering to form the wall, these precursors have to be ligated on cell surface by nisin since it binds with itself firmly at lipid II also stop building of same (Fig 26). This inhibition weakens the cell wall, leading to osmotic stress and lysis of bacteria.[11] Diagramatic MOA of Inhibition of Cell Wall Synthesis Shown in Fig.3.

C) Interference with Essential Enzyme Functions

Inhibiting important enzymes within the bacteria - Bacteriocins can also inhibit essential enzymatic functions inside of a bacterial cell. For example, some bacteriocins can block the enzymes associated with DNA replication or RNA and/or protein synthesis. Through inhibition of these enzymes, bacteriocins disrupt essential metabolic processes creating an integrated response that results in bacterial growth and cell death.[12] Diagramatic MOA of Interference with Essential Enzyme Function Shown in Fig.4.

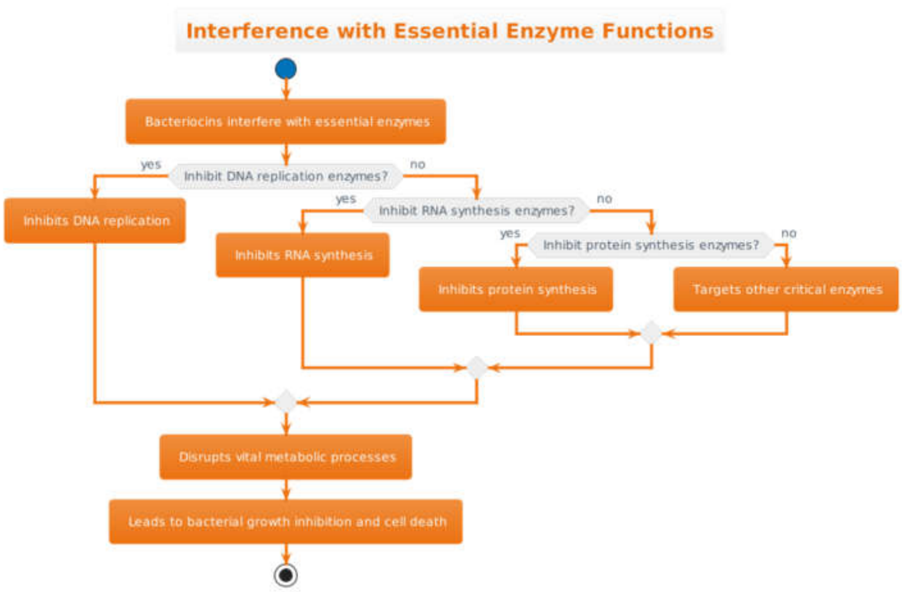

Fig.4: Interference with Essential Enzyme Functions

D) **Induction of Apo ptosis-like Processes:**

Some bacteriocins may initiate apoptosis (a programmed cell death indicative of eukaryotic cells); This pathway enables the discharge of set paths that drive programmed cellular disintegration and cell death. Although this mechanism of action has not been as thoroughly dissected in comparison to the others, it represents an exciting area for future research and potentially new means of antbacterial targeting**.**

E) Additivity Synergistic effects with other antimicrobials

A bacteriocin may show synergy effect with other antimicrobials where the combined use of both is greater than individual one. There are several ways this synergy takes place, such as:

⇓ Permeabilization: Bacteriocins can disrupt the cell membrane, which may improve targeting of other antimicrobials to enter the cytoplasm.Both Bacteriocins and other uses of antimicrobials can attack different cellular processes, resulting in dual targeting attacking the same bacteria at more fronts. [13] [14] DiagRAMATIC MOA of SYNERGISTIC EFFECTS WITH ING ANTIMICROBIAL SHOWN Fig. 5

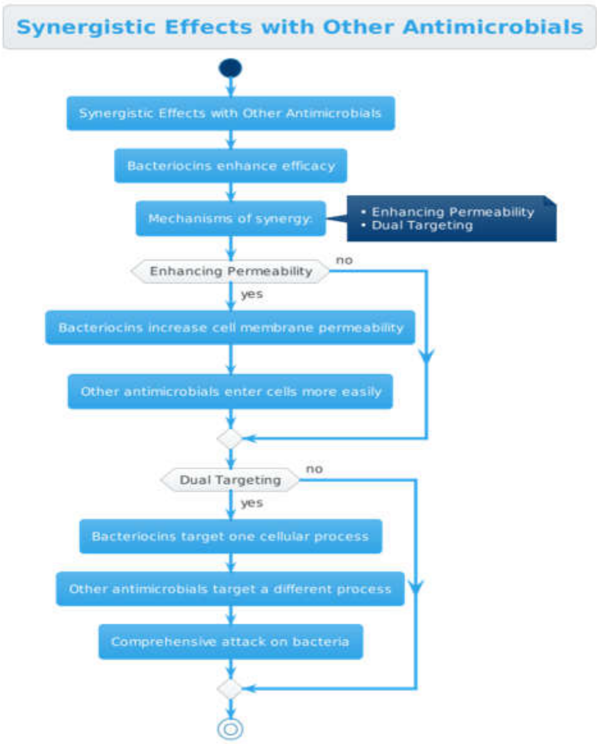

Fig.5: Synergistic Effects with Other Antimicrobial

LACTIC ACID BACTERIA

The majority of lactic acid bacteria (LAB) are Gram-positive, non-spore-forming cocci or rods that are part of the fermentation metabolic pathway. A small percentage of LAB are also catalase-negative. This is significant for the food industry and human health because they involve in fermentation as well as posses probiotic properties.[15] LAB strains are considered as fast-growing microorganisms of great interest for the inspection of different metabolic pathways in prosaic conditions. Many of these metabolic pathways produce a variety of other useful molecules that the restoredsynthetic producer cells can use to convert into more basic, desirable building blocks-like organic acids/aminoacetones/terpenes or even antimicrobial compounds and special enzymes capable of breaking down complex organics. [16] has constituted the basis for present classification of lactic acid bacteria (LAB) according to following characteristics: growth temperature, glucose fermentation pathway used by strains presence and/ or form of a cellular envelope components involved in sugar transport. LAB included the following taxa; Lactobacillus, Leuconostoc, Pedidiococcus and Streptococcus.[17] The vast majority of LAB belong to the order Lactobacillales, class Bacilli, and phylum Firmicutes. The order Lactobacillales contains about

30 genera, over 300 species (and still growing with the discovery of new ones), and includes six families: Aerococcaceae, Carnobacteriaceae; Enterococcaceae; Lactobacillaceae; Leuconostocaceaem and Streptococcaceae.[18] The classification of LAB has been primarily based on the gram reaction and generation of lactic acid from diverse fermentable carbohydrates.[19]

LACTIC ACID BACTERIA ISOLATION

Specific strains of lactic acid bacteria were directly tested from fermented milk, and samples of cheese were homogenised using saline solution, serially diluted, and then plated on MRS agar. To detect lactic acid bacteria, a few colonies were randomly chosen from the plates containing MRS agar after 48 hours of incubation in an aerobic environment at 37°C. The separated colonies were cultivated on MRS slants, incubated for 48 hours at 37°C, and maintained at 4°C until testing. [20]

APPLICATION OF BACTERIOCIN

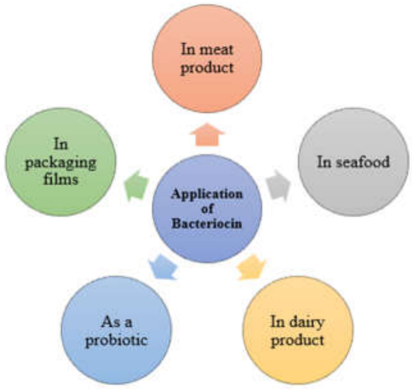

Fig.6 : Application of bacteriocin

Consumer awareness of the necessity of natural preservatives—which pose no risks to human health—is growing. Include the Use of Bacteriocin in Fig.6.

Table 1:- Examples of bacteriocin in food preservative.

Name of bacteriocin	Application	Advantage of using them	Reference
Nisin A	The method of meat binding includes nisin.	Nisin prevents unwanted bacteria from growing in beef products.	[21]
Enterocin 4	Aspergillus faecalis In addition to producing enterocin, INIA4 acts as a starter culture for Manchego cheese.	L. monocytogenes development is suppressed by Enterococcus faecalis INIA4. Ohio, but not Leptospirosis. Scott A.	[22]
Pediocin AcH	Munster cheese is treated with pediocin from Lactobacillus plantarum (WHE 92) during the first stages of ripening.	The spray can be used as an antilisterial treatment since it prevents L. monocytogenes from growing.	[23]
Pediocin	The yeast Saccharomyces cerevisiae expresses optonisin.	Usually, baked items and wine are preserved with it.	[24]
Pediocin PA-1	It is frequently utilised as a culture starter for banger fermentation.	It mostly prevents L. monocytogenes from growing.	[25]

Leucocin A	It is used to stop meat from deteriorating.	Lactobacillus sake's shelf life can be extended by up to nine weeks when vacuum-packed meat is injected.	[26]
Lactocin 705	It mostly prevents L. monocytogenes from growing in ground beef.	It mainly prevents Listeria monocytogenes from growing in ground beef.	[27]
Piscicolin 126	The main purpose of it is to manage L. monocytogenes in devilled gammon paste.	As compared to other bacteriocins, it is more effective.	[28]

In meat products: Infection and Food Safety: Bacteriocins are able to eliminate foodborne pathogens, being more active against Listeria monocytogenes in cooked meat products than a pathogenic strains of Staphylococcus aureus and the enterotoxic strain 0157 H- Escherichia coli lineages. This will go a long way in ensuring the safety of these products.[29] Bacteriocins are antimicrobial peptides that assist extend the shelf life of meat products by inhibiting bacterial spoilage..[30] Being of natural origin, bacteriocins are a potential choice for chemical preservatives. They considered GRAS (generally recognized as safe).[31] Bacteriocins preserve the quality of meat products in terms of their sensory properties (definition as above) but without spoilage. Using bacteriocins in conjunction with your preservation techniques (MAP or refrigeration) can increase their effectiveness.[32], [33] Nisin is also a useful agent in cold meat binding system because it can inhibit growth of Brochothrix thermosphacta.[34] Eg. of Bacteriocin in Food Preservative Shown in Table.1.

In Seafoods: Fishery : They may be added to the fired fish from the shooting or directly placed in from sea food solutions. According to some research, using vacuum and protective cultures along with bacteriocins can stop L. monocytogenes from growing (Table 1). pressed cold-smoked salmon. This study investigated inhibition of Listeria monocytogenes K1-101 by sakacin P in cold-smoked salmon and the production of sakacin P from four silked cultures. The salmon samples that had been vacuum-packed were kept at 10° C for four weeks. [35] Pathogens: Research has shown that these compounds have inhibiting effects against several key foodborne pathogens commonly associated with seafood such as Listeria monocytogenes, Vibrio spp., and Clostridium botulinum. It reduces the likelihood of foodborne illness.[36] Gentle Handling: Obviating the need for high-heat processing, as with most shells and ready-to-eat products helps to maintain The nutritional profile of fish and retains its sensory properties. This is more relevant in case of sushi and sashimi, for these are high-value products.[37] Other methods, like bacteriocins with refrigeration or gas packaging (MAP), and high-pressure processing (HPP) can be combined to potentiate the antimicrobial effect.[38]

In Dairy products: L. monocytogenes was documented with an other reason than the general mentioned before, however this microorganism is related to multiple outbreaks mediated by dairy products such as pasteurised milk and cheese. [39] Nisin's efficacy against L. monocytogenes in dairy products has also been demonstrated. [40]

In Probiotic: Many probiotics and other bacteria create antimicrobial peptides called bacteriocins, which prevent the development of related or similar bacterial strains.

Improved Gut Health: Bacteriocins can combat pathogenic bacteria which in turn supports the beneficial microbes residing inside your gut. This is especially advantage in prevention and management of gut infections.[41] By eliminating harmful bacteria, Bacteriocins produced by probiotic strains further extend shelf-life of food applications and are used as a natural preservative in various products.[42] Bacteriocins-Probiotics Producing Strains Taking into account those studies, we propose that bacteriocinogenic probiotic bacteria could be considered as complementary strains for nah+ better protection against infectious diseases. Human intestines coexist with intestinal bacteria and the host. The other way around, the microflora in stomach acts as a trigger for development of precursors to mucosal immune system.

Bacteriocins in packing film:Research on using bacteriocins in packing films to prevent food spoiling and harmful organisms has been ongoing for the past ten years. When antimicrobial packaging film comes into close touch with the surface of goods like cheese and meats, it stops microorganisms from growing on the food.

Because of this, in order for the antimicrobial packaging film to be effective, it needs to come into touch with the food's surface to allow the bacteriocins to permeate it. There might be a benefit to bacteriocins being released gradually from a packing film onto the food surface as opposed to dipping and spraying meals with the substance.

CONCLUSION

The intercontinental transportation of food has made efficient and fast preservation methods necessary in order to maintain both nutritional quality worldwide, while simultaneously minimising the risk for human health. We are using modern preservation techniques such as pasteurisation, canning and use of chemical preservatives to supplement or replace traditional methods (curing with salt, free air drying). Safety issues related to chemical preservatives have prompted biopreservation methods, notably the use of bacteriocins (antimicrobial peptides synthesized by bacteria). Based mainly on the structure and action mode, Three primary classes comprise bacteriocins: Class I (lantibiotics), class II (non-lantibiotics). Based on size of protein molecule having it also be classified as a sub-group called large heat-labile proteins.Class III. They exert their effects by disturbing cell membranes, preventing cell wall synthesis, ritarding or killing vital enzyme reactions and even provoking apoptosis-like process in bacteria. Nisin, a well-studied and commercially prevalent antimicrobial produced by lactic acid bacteria (LAB), is among such acute toxins. LAB, a probiotic organism genus including Lactobacillus, Leuconostoc and Streptococcus; LAB are crucial in conditions of food fermentation and health with benefit of their antibiotic properties (e.g., bacteriocins). Since then, these bacteriocins have been employed in a number of food products such as meat and seafood; they were also incorporated into dairy items.while some reports elaborates their use even in packaging films aimed to extend shelf tables without significant concern on health or safety issues from human intervention. Certain key points such as broad- spectrum activity, stability under different storage conditions, food matrix compatibility and cost of production play a significant role in the selection of bacteriocin candidates for their use as preservatives in foods. The growing consumer demand for natural preservatives makes bacteriocins a promising alternative to chemical preservatives, offering a safer and effective means to maintain food quality and safety.

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