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Bacteriocin as a bio-preservative and its applications in food products

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Abstract

Bio-preservation is the technique of prolonging the shelf-life of food with the use of either synthetic or natural antimicrobials or controlled microbiota. Lactic acid bacteria primarily produce bacteriocin, organic acids, and hydrogen peroxide. Proteins or peptides with antibacterial properties make up bacteriocin. In accordance with its dimensions, structure, and post-translational alterations, bacteriocin is classified into four different groups. Due to their wide range of bactericidal action, non-toxicity, immunogenicity, and thermos resistance, lactic acid bacteria bacteriocins are regarded as efficient bio-preservative agents. A vast number of gram-positive and gram-negative bacteria produce the substance known as Bacteriocin. It has a protein structure and acts as either a protein or a polypeptide to kill microorganisms. Antibacterial peptides or proteins termed bacteriocin are created by the ribosomes of bacteria. Bio-preservation, which is produced by lactic acid bacteria, is the process of extending the shelf life and food safety by the use of natural or controlled microbiota and/or an antibacterial agent. One of the most common techniques of food bio-preservation is fermentation, which is based on the development of bacteria in food, whether they are added naturally or not. It is advised to use Bacteriocin to prevent the formation of dangerous germs because it is food-grade, more natural, beneficial to health, and popular with the general public. Based on its properties, structure, molecular weight (MW), and antimicrobial spectrum, bacteriocin is classified into three major categories: antibiotics and non-antibiotics with low MW and those with greater MW. Bacteriocin can be separated and purified using many techniques. This review article highlights the bacteriocin uses in food, agriculture, animal husbandary and pharmaceuticals industries. It also focuses on how bacteriocin regulates food pathogenic bacteria in foods to extends the shelf life of food products.

Keywords: Bacteriocin, bio-preservation, lactic acid bacteria, purification, shelf life

Introduction

Meals made from animals are especially perishable due to their high nutritional content, high moisture content, and neutral pH. For these products to remain safe and of high quality, comprehensive preservation is essential. Bio-preservation is a way to preserve food which makes use of the antibacterial properties of naturally existing organisms and their metabolites. It is capable of balancing modern concerns for food safety and quality with traditional means of food preservation. The efficiency of biological antimicrobial systems, such as lactic acid bacteria (LAB) and/or their bacteriocins, bacteriophages, and bacteriophage encoded enzymes, is greatly influenced by the bio-preservation techniques used to preserve various foods. In the food business, they are usually employed to obtain the typical flavour and texture of the food products. They are a well-known type of bio-preservation that is often used in the food industry in developed countries. These are typical bio-preservatives found in the industrial nations that support preserving the food's safety and nutritional worth. The main issue facing the food sector nowadays is a product's shelf life, which is frequently shortened by illnesses. There are a few additional ways to manage these bacteria, including heating, freezing, chemical preservatives, etc. The development of bio-preservation methods, which notably utilise bactericidal agents, is a result of advancements in the science of food preservation, which have occurred considerably more recently. Despite being produced by lactic acid bacteria and possessing an antimicrobial effect, bacteriocins are not considered to be real antibiotics. Therapeutic antibiotics elicit allergic reactions, whereas bacteriocins do not in certain persons. Bacteriocins differ from clinical antibiotics in that they are proteinaceous by nature and are swiftly broken down by the digestive enzyme protease in people. These principles make it simple to avoid misunderstanding bacteriocins with antibiotics. Proteins called bacteriocins have both physiological activity or complexity and antimicrobial effects on other bacteria, usually those that are closely related to them.

They are ribosomal synthesis-produced bactericidal polypeptides. The highly diversified class of bacteriocins is often chosen for research and application as specific antagonists against diseases and pathogenic bacteria. The use of bacteriocins can occasionally be limited for a number of reasons, and their high price remains a significant hurdle to their wider adoption. Gram-positive and Gram-negative bacteria both produce substantial amounts of bacteriocins, which are substances with a protein structure (either proteins or polypeptides). The variants of species that are related to the organisms that generate the bacteriocins, and particularly the strains of the same species, are the only ones that can produce them. The primary difference between bacteriocins and antibiotics is this. Antibiotics, on the other hand, have a wider range of activity and, even when restricted, do not have a preferred influence on strains that are closely related to one another. Furthermore, bacteriocins are produced during the main phase of development through ribosome synthesis, while antibiotics are usually secondary metabolites ^[1]. Since bacteriocins typically have modest molecular weights (rarely exceeding 10 kDa), undergo posttranslational modification, and are easily decomposed by proteolytic enzymes, particularly those present in the mammalian gastrointestinal system, they are regarded as safe for eating.

Bio-preservation

The process of bio-preservation involves employing naturally produced or inadequately controlled microbes and/or their antimicrobial compounds to lengthen the safety and storage stability of food ^[2]. One of the most popular techniques for food bio-preservation is fermentation, which is based on the growth of microbes in foods, either present naturally or added. Most of these species were lactic acid bacteria, which generate organic acids and other substances that give food distinctive flavours and textures in addition to their antibacterial qualities. Natural fermentation processes have historically been used to maintain a variety of foods from deteriorating. Currently, fermented foods are increasing in popularity and account for 60% of diets in advanced economies ^[3]. Starter cultures for fermented products are preparations of one or more organism systems that are used to initiate the fermentation process during food manufacture ^[4], mainly in the dairy industry, and more recently to other cultured foods like meat, alcoholic drinks, vegetables, and juices. In order to positively impact the physical, chemical, and biological composition of foods and provide sensory attributes that consumers find appealing; the bacteria employed are chosen based on the type of food. Microorganisms must have GRAS status (Generally Recognized as Safe by Humans and the Scientific Community) and should not already be known to be harmful or hazardous in order to be used as starting cultures.

Lactic acid Bacteria (LAB)

Lactic acid bacteria (LAB) were recognised as a class of bio-preservative bacteria at the turn of the 20th century as a result of their involvement in the food laboratory. LAB is necessary for food fermentation. Food preservation, the creation of aroma compounds, and microbiological stability are all becoming more and more dependent on LAB. ^[5] The species that are collectively referred to as the "LAB" group of bacteria are *Lactococcus*, *Streptococcus*, *Lactobacillus*, *Pediococcus*, *Leuconostoc*, *Enterococcus*, *Carnobacterium*,

Aerococcus, *Oenococcus*, *Tetragenococcus*, *Vagococcus*, and *Weisella*. Several rod- and coccus-shaped, gram-positive, non-spore-producing, non-motile microorganisms can all ferment carbohydrates. The main natural result of fermentation is lactic acid. The most common application of LAB is in the fermentation of GRAS-recognized foods (generally recognised as safe). A variety of fermented dairy, meat, and vegetable products can be produced using LAB. It helps keep food's nutritional value intact, increases its shelf life, and makes an effort to protect it against pathogenic organisms and food deterioration. Hydrogen peroxide, bacteriocins, and organic acids are the end products of LAB fermentation. Probiotics can also be produced using LAB. The genera *Lactobacillus* and *Bifidobacterium* have a substantial impact on human gastrointestinal tracts, making them particularly important in this research. Other probiotic species include *P. acidilactici*, *L. mesenteroides*, *S. thermophilus*, *L. lactis* subsp. *lactis*, *E. faecalis*, and *E. faecium*.

Bacteriocin

Antimicrobial peptides or proteins termed as bacteriocins are generated by microbes using their ribosomes. Colicins, which are typically quite large proteins that may weigh up to 80 kDa and are largely generated from *E. coli*, are the principal bacteriocins that were initially described. When they adhered to the inner membrane or other cytosolic targets, they demonstrated the ability to destroy extremely closely related bacteria ^[6]. Furthermore, bacteriocins, which are small, heat-stable cationic peptides produced by Gram positive bacteria and have a wider range of inhibitory activities, are now widely known ^[7]. LAB bacteriocins have traditionally been the backbone of food bio-preservation due to their reputation as being safe for use in food.

LAB Bacteriocin

Bacteriocins are protein-based toxins that certain bacteria make to inhibit the development of other, similar, or closely related bacteria ^[8]. The ribosome produces physiologically active peptides called bacteriocins, which have antibiotic effects on other bacteria, mainly closely related species. Unlike other antibiotics, bacteriocins are proteinaceous in their natural environment, and the human gastrointestinal tract's proteases efficiently break them down. Because they are produced through ribosome synthesis, bacteriocins can have their characteristics altered to increase their effectiveness and range of activity ^[9]. According to ^[10], the LAB bacteriocins are a worthy contender for application as food preservatives since they have the following beneficial qualities:

- Also, while proteins break down, the digestive system's proteolytic enzymes successfully prevent this process.
- Generally immunogenic and suitable for use in lab animals.
- Totally ineffective in eukaryotic organisms.
- Common thermos resistance, which refers to an antibacterial agent's capacity to continue working even after pasteurisation and sterilization.
- The broad bactericidal effect has an impact on a wide range of organisms, including *Listeria monocytogenes*, *Bacillus cereus*, *Staphylococcus aureus*, and *Salmonella*. Furthermore, it affects the majority of Gram-positive bacteria in addition to specific damaged Gram-negative

bacteria.

- Even before chromosomes contain a large number of genetic determinants, genetic modification can be used to increase the number of natural peptide analogues with acceptable properties.

Because of this, the use of bacteriocins as bio-preservatives in food has recently received a lot of interest in an attempt at producing peptides with ever-increasing potential. Although a cytolysin manufactured by *Enterococcus faecalis* has recently been found as possessing both hemolytic anemia and bacteriocin activity, the cytolytic capacity of bacteriocins is presently an important problem [11]. With the use of recombinant DNA technology, manufacturing can now be increased, bacteriocin genes can be introduced into novel species, and bacteriocin variants with much enlarged and/or more active spectra can be determined and transformed [12].

Classification of LAB Bacteriocin

Due to their development and specific effects against species of bacteria, the characterization of the multiple Bacteriocins that LAB develops over time have diverged from one another. The majority of bacteriocin molecules include 20-60 residues of amino acids and are cationic, hydrophobic, or amphiphilic. For each of the three primary classifications, bacteriocins are further subdivided down into subcategories. discusses how several bacteriocin classes came to be [13] divided the four primary antimicrobial peptide or protein classes of LAB bacteriocins (bacteriocins).

- Lantibiotic.
- Short, hydrophilic, high - temperature peptide (13 kDa).
- A large (30 kDa) high - temperature protein.
- Complicated proteins that require substantial lipid or carbohydrate molecules in order to have an antimicrobial property.

Category I (Lantibiotics)

The uncommon thio-ether amino acids lanthionine and -methyl lanthionine as well as other modified amino acids, such as gene-encoded serine and threonine, would belong to a classification of membrane-active peptides known as lantibiotics. Dehydroalanine and dehydrobutyrine are really the products of the enzymatic dehydration of these amino acids, respectively [14]. Enzymatic dehydration can be utilized to, respectively, create dehydroalanine and dehydrobutyrine [14]. Less than 5 kDa in size, they are microscopic, heat-stable peptides that interfere with membrane structure [15]. Nisin is the strongest component of this ensemble. It is possible to identify post-translationally changed amino acids by their residues. The nisin made by *Lactococcus lactis* subsp. *lactis* is the greatest example of this class. Ia and Ib are the additional subcategories of Class I. Nisin is a representative of the class Ia of bacteriocins, and it is primarily made of cationic and hydrophobic peptides that penetrate the target membranes and have a more extensible structure than class Ib. Class Ib bacteriocins work by preventing vulnerable bacteria from carrying out crucial enzymatic operations [17]. They have an equilateral sphere and no net negative charge.

Category II (small, heat-stable peptides)

The majority of the recently discovered bacteriocins are Category II bacteriocins, which are normally unaltered, relatively small (30-100 amino acids, 10 kDa), and heat-

stable. It has been found that whereas the overwhelming of bacteriocin producers only synthesise one bacteriocin, certain LAB create different bacteriocins (2-3). It has been determined that a signalling pathways system composed of an induction factor (IF), a histidine protein kinase (HK), and a response regulator (RR) tightly regulates the transcription of the production of some class II bacteriocins (plantaricins of *Lactobacillus plantarum*C11 and sakacin P of *Lactobacillus*'s sake) [10]. Class IIa includes Pedicin PA-1 [18]. Besides that, anti-listerial pharmaceuticals with conserved N-terminal action scenes including Sakacin P. At the N-terminus of the peptide, two cysteines connect by an S-S bridge.

Class IIb: Bacteriocins are peptides that are created by combining two different, independent-sequence peptides. Examples include lactococcin G and plantaricins EF and JK. Both peptides need to be completely active for this to function. The peptides' initial amino acid sequences differed. One gene is acceptable, despite the fact that each immune gene is encoded by its own surrounding genes [19].

Class IIc: Short, high - temperature peptides are transferred via leader peptides. Acidocin B and divergicin A are also these.

Category III (Large Heat labile Bacteriocins)

These specific large peptides are heat-labile proteins with molecular weights greater than 30 kDa. There are also lactacins A and B, shelveticins J [20], and helveticins V [21] in it. At pH 7.0, the majority of low molecular weight bacteriocins are substantially cationic, a feature that both lantibiotics and non-lantibiotics have seemed to share [22].

Category IV (Circular peptides)

Bacteriocins, which build up into sizable chemical complexes with other molecules, as well as any lipids or carbohydrates essential for activity, make up the majority of these substances. As it is thought that these bacteriocins generate compounds with other biomolecules in the crude extract as a result of their cationic and hydrophobic properties, they have not yet been identified. Microorganisms attached to food produce a large percentage of the bacteriocins, which are categorized into Classes I and II.

Purification of Bacteriocin

lactic acid bacteria from various sources, such as plant material, food stuffs, human or animal isolates, are checked as the very first phase in the separation of bacteriocins. Many lactic acid bacteria species, among which are those that cause tainted milk and cheese, have been found by [23]. The well diffusion experiment on agar plates was employed in order to identify the antagonistic action [24]. Catalase also added to the growth medium to try and counteract the inhibitory effects of hydrogen peroxide, and phosphate buffer was added to the solid media to try and counteract the inhibiting adverse reactions caused by organic acids. It may be necessary to undertake more extensive research in a liquid medium to establish the minimal inhibitory concentration. Although bacteriocins produced by lactic acid bacteria have been extensively studied recently, little is known about the chemical makeup of these compounds. This might be because these antimicrobial peptides are difficult to purify [25]. Several techniques have been developed to remove bacteriocins from

complex culturing broths using their cationic and hydrophobic properties [26]. Bacteriocins' propensity for organic solvents, fluctuation in solubility in concentrated salt solutions, and at a general and particularly acidic pH level are commonly taken into consideration while developing extraction techniques. But since hydrophobic interaction between the bacteriocin molecules and the bacterial cells is what causes bacteriocins to inactivate microorganisms, the presence of hydrophobic regions in bacteriocin molecules is important for their efficacy against sensitive bacteria [27]. Bacteriocins can be homogeneously purified by LAB using three different techniques. You might start with a traditional method of purification that requires a time-consuming set of subsequent procedures such as ammonium sulphate precipitation, ion exchange, hydrophobic interaction, gel filtration, and reversed-phase high-pressure liquid chromatography [28-29]. Moreover, a straightforward three-step approach has been created [30], includes I precipitation of ammonium sulphate, (ii) extraction and precipitation of chloroform/methanol, and (iii) other techniques, in addition to reversed-phase high-pressure liquid chromatography, which is the only chromatographic technique employed. Finally, after raising the accessible bacteriocin titer by altering the pH of the crude fermentation medium, bacteriocins can be isolated utilizing a unique unit operation, namely expanded bed adsorption using a hydrophobic interaction gel [30-31]. The last two approaches have been used to purify a variety of bacteriocins with fascinating promise for advanced manufacturing. They are quicker than the first traditional method while still being effective. Many enterocins (made by *Enterococcus faecium* RZS C5, RZS C13, and FAIR-E 406 strains), the class II Bacteriocin amylovorin L (produced by *Lactobacillus amylovorus* DCE 471), and various other bacteriocins are among them (produced by *Streptococcus macedonicus* ACA-DC 198).

Application of Bacteriocin

As more than just a response, bacteriocins are commonly used in a variety of industries, but the food preservation industry stands to gain the most from their application. In-depth investigation on the use of bacteriocins in the foodservice industry has focused on dairy, egg, vegetable, and meat products. Nisin A, in conjunction with its natural counterpart, Nisin Z, is one of the LAB bacteriocins that has been found to be highly effective against the microorganisms that can cause food illnesses and spoilage. Nisin, which has previously acquired general permission for usage, is the only bacteriocin that has been authorized utilized in the food-processing sector [32-17]. Several more preserving methods have been employed in order to prevent damaged food and food-borne infections. The application of heat (pasteurization, thermal sterilization), lowering pH and water activity (acidification, dehydration), and incorporating preservatives are a few of these procedures (antibiotics, organic compounds such as propionate, sorbate, benzoate, lactate, and acetate). Despite the reality that these strategies have been demonstrated to be highly effective, there is an increasing desire for organic, microbiologically safe products that provide customers significant health benefits. In contrast to being used as an ingredient in food processing, bacteriocins may additionally be introduced to a product following its previous fermentation fermented with one by adding other bacteriocin-producing strains (starter culture). There are various applications for bacteriocins in the food,

medicinal, and agricultural sectors.

Preservation of Food

The preservation of food has previously made considerable use of bacteriocins. Many investigations have examined the use of bacteriocins in the food sector, particularly in dairy, egg, vegetable, and meat products. The FDA-approved Nisin has been utilized in over 48 countries, and Nisaplin TM is advertised as a natural food preservative. It inhibits the development of a range of Gram-positive bacteria in many food systems, including several significant food-borne diseases like *Listeria monocytogenes*. It primarily defends against heat-resistant spore-forming organisms like those of the genera *Bacillus* and *Clostridium* and is particularly effective when used in the production of processed cheese and spreads. This is necessary for avoiding *Clostridium botulinum* infections since the toxin that this species generates may have significant consequences. Other bacteriocins, including lacticin 3147 and lacticin 481, have not yet seen widespread commercialization but have the potential to be used as natural preservatives and flavor enhancers. *Listeria monocytogenes* is effectively eliminated by Pediocin PA-1, a broad-spectrum lactic acid bacteriocin used as a food preservative [33]. Using a purification or semi-purified bacteriophage preparation as a food component, combining an ingredient that has formerly been fermentation with a strain that produces bacteriocin, or having supplemented all or a portion of the starter culture in fermented foods with a bacteriocin-producing culture to produce bacteriocin in situ are the three least dangerous ways to add bacteriocins to food [17]. Bacteriocins can also be utilized to improve cheese's gas-blowing fault or speed up proteolysis to improve food's sensory attributes. Bioactive packaging, which effectively shields food from outside pollutants and lengthens food shelf life, is another use for bacteriocins [34].

Application of bacteriocin in seafood products:

LAB with in food manufacturing is of particular interest to producers of Gram-positive and Gram-negative bacteriocins because these bacteria are generally perceived to be safe. Several of the bacteriocins produced by lactic acid bacteria have been approved for use in food. Pediocin PA-1 and the group of nisin generated by *Pediococcus acidilactici* are the only bacteriocins now used in commerce [35]. Employing bacteriocins for food bio-preservation has generally comprised three strategies [36].

1. Food is given an injection of LAB, which produces bacteriocin in the final product. The LAB must be capable of producing bacteria and generate bacteriocin in the goods for them to be used appropriately.
2. Employing pure or synthetically produced bacteriocins as a food preservative.
3. Applying bacteriocin as a food additive to a product that has previously conducted fermentation with such a strain that is capable of producing it.

Application of bacteriocin in dairy products

Cell lysate should be included in the initial culture for better flavor development. The proportion of nisin used depends on the meal's composition, the number of spores present, the needed shelf life, and the storage temperature. Other bacteriocins that have recently been researched in milk and dairy products include lacticin 3147, which is beneficial

against unfavorable LAB, *L. monocytogenes*, *S. aureus*, and *E. coli* O157:H7 in milk, as well as pediocin AcH, which is effective against unfavorable LAB, *L. monocytogenes*, *S. aureus*, and *B. cereus*. Nisin-containing cheddar cheese produced by lactococci that produce nisin is utilized as a component in pasteurized process cheese or cold pack cheese spreads to combat *B. cereus*, *S. aureus*, and *Listeria monocytogenes* in milk and Manchego cheese. The nisin-containing pasteurized processes cheese used to have a significantly more prolonged shelf life than the control cheese spreads (301 and 387 IU nisin/g) [37]. Bacteriocins are frequently used in the milk sector, especially when the products are fermenting. Several studies have shown that nisin is safe against harmful bacteria including *L. monocytogenes* found in *Camembert cheese* and *Clostridium botulinum* found in cheese, as well as the strains that create it. It is important to investigate if bacteriocins with lytic properties, such as nisin and lactic in 3147, can be used to accelerate the ripening of cheddar cheese.

Application of bacteriocin in canned food products

Alcoholic beverages: Nisin is a substance that can be used to prevent wine or beer from going bad since yeasts are unable to utilize it. It can continue to function during fermentation without impairing the flavor, growth, or fermentative performance of brewing yeast strains. It could therefore be used to extend the shelf life of the beer and reduce the pasteurization time-temperature combination [38]. In order to limit bacterial degradation, nisin may also be used to reduce the amount of sulphur dioxide required in the manufacture of wine [39].

Application of Bacteriocin in fish products

Gram-negative microbes are typically too responsible for the deterioration of fresh fish, despite the fact that pathogenic species like *Clostridium botulinum* and *Listeria monocytogenes* can sometimes cause issues with vacuum-packed fresh fish and shellfish. Moreover, Nisin and Microgard prolonged the shelf life of fresh chilled salmon, decreased the numbers of all aerobic bacteria, and even stopped the growth of *L. monocytogenes* inoculated into frozen thawed salmon [40]. More subsequently, [42] showed the impact of LAB cultures on the management of fish pathogenic bacteria [41] showed the significant effects of lactic acid, sodium chloride, and/or nisin in rainbow trout. *Listeria* is prevented in cold-smoked salmon with *L. sake* or *C. divergens*.

Application of Bacteriocin in meat products

It is essential that you recognize that meat and meat products are complex systems with a multitude of variables affecting the development of microorganisms and metabolite production. This is important when investigating a bacteriocin-producing culture for sausage fermentation and/or bio-preservation. Therefore, it is crucial to evaluate how fermentation and formula technologies enhance the functionality of bacteriocin-producing cells. The most well-known bacteriocins discovered in meat as well as meat-related products include nisin, enterocin AS-48, enterocins A and B, sakacin, leucocin A, and and even more so pediocin PA-I/AcH. Those certain bacteriocins are additionally employed in conjunction with a variety of physicochemical processes, such as modified atmosphere packaging, high hydrostatic

pressure (HHP), heat, and chemical preserve in an effort to stop the spread of these illnesses, certain bacteriocinogenic LAB have been utilized as bio protective cultures for food processing activities. In accordance with the literature that is generally available, it is unclear exactly nisin can be utilized in fermented and cured beef. Nisin being used in meat products has not been as beneficial as it has been in dairy products due to its poor solubility, unequal distribution, and lack of stability. Nisin is not an indigenous meat strain, despite the fact that pediocin PA-I/AcH is more appropriate for usage with meat and animal products [37].

Conclusion

According to its extensive useful applications in the pharmaceutical and food sectors, bacteriocin from generally accepted to be secure (GRAS) LAB has continued to attract the attention of an increasing number of research organizations. It has demonstrated antimicrobial properties against harmful microorganisms. Bacteriocin is a typical bio-preservative used in the industrialized world to keep food safe and pure microbiologically. Nisin constitutes the sole bacteriocin which is employed industrially and is sold. Bacteriocin has supposedly been used to address issues about food rotting and food-borne illness.

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