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THE ROLE OF BACTERIOCINS IN THE CONTROLLING OF FOODBORNE PATHOGENS

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ABSTRACT

Bacteriocins are antimicrobial peptides or proteins produced by strains of diverse bacterial species. The antimicrobial activity of this group of natural substances against food borne pathogenic, as well as spoilage bacteria has raised considerable interest for their application in food preservation. Depending on the raw materials, processing conditions, distribution, and consumption, the different types of foods offer a great variety of scenarios where food poisoning, pathogenic, or spoilage bacteria may proliferate. Application of bacteriocins may help reduce the use of chemical preservatives and/or the intensity of heat and other physical treatments, satisfying the demands of consumers for foods that are fresh tasting, ready to eat, and lightly preserved. In recent years, considerable effort has been made to develop food applications for many different bacteriocins and bacteriocinogenic strains. Antibacterial metabolites of lactic acid bacteria have potential as natural preservatives to control the growth of spoilage and pathogenic bacteria in food. Among them, bacteriocin is used as a preservative in food due to its heat stability, wider pH tolerance and its proteolytic activity. Due to thermo stability and pH tolerance. it can withstand heat and acidity/alkanity of food during storage condition. Bacteriocin are ribosomally synthesized peptides originally defined as proteinaceous compound affecting growth or viability of closely related organisms. Hence, issues an overview of bacteriocins and their applications in food preservations are addressed in this review.

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INTRODUCTION

For the past decades, food safety and food control have been an important issue in many countries. According to a report of the World Health Organization, hundreds of millions of people worldwide suffer from food borne diseases (1). Food borne diseases cause approximately 76 million illnesses, resulting in 325,000 hospitalizations, and 5,000 deaths each year in the US alone. Diarrhoeal diseases, mostly caused by food borne microbial pathogens, are leading causes of illness and deaths in the developing countries, killing an estimated 1.9 million people annually at the global level (2).

Presence of bacteria in minimally processed foods raises concern about products safety and quality. Food borne pathogens as well as spoilage microorganisms may multiply in these products during extended refrigerated storage, thus threatening consumer's safety. These microorganisms are abundant in the environment and are naturally present in human and animal intestine. Crosscontamination during slaughtering and carcass processing has a significant effect on the microbiological quality of meat (3).

To inactivate food borne pathogens, novel technologies such as biopreservation systems, non thermal technologies, or combined treatments have been studied. Among biopreservatives, bacteriocin has caught the attention of food scientists to be used as a natural food bio-preservative due to its antimicrobial activity against food spoilage and pathogenic bacteria (5). Use of lactic acid bacteria or their antagonistic metabolites such as lactic acid, hydrogen peroxide and bacteriocins is an example of biopreservation. Lactic acid bacteria (LAB) are generally regarded as safe as they have been associated with production of fermented foods in many centuries. For this reason, these bacteria are attractive as a means of naturally controlling growth of pathogenic and spoilage organisms in a variety of foods. Bacteriocins of LAB are known to be bactericidal to closely related gram positive bacteria and few reports are available on their activity against gram negative microorganisms due to outer membrane acting as a permeability barrier (4).

Use of microorganisms in food fermentation is one of the oldest method for producing and preserving food. Much of the world depends upon various fermented food that are staples in the diet. Till the end of 1950, a very few of the food items were processed and packaged. Processed and packaged foods were luxury item in colonial times but after 1960 these food items were in great demand globally due to growing urbanization, breakdown of large families into nuclear families and increase in the number of working women. Chemical preservatives and other traditional barriers have been used in food products to inhibit microbial growth which lead to serious health disasters, thus challenging the food scientists for providing safer and healthier food. Food preservation has become a major issue because food borne pathogens can cause havoc in preserved/fresh food items at high temperature, room temperature and even at low temperature (6).

However consumer demand for faster, healthier and ready-to-eat products have strongly demanded the use of more natural preservatives instead of chemical preservative. Microbiologists around the world got interested in bacteriocin-producing microorganism to overcome these problems that fulfill the requirement of food preservation. Although many other type of bacteriocin such as subtilin, cerein, thuricin, plantaricin etc have been isolated and characterized and are still in a process of getting commercial status to be used as food preservatives , so far only one bacteriocin, Nisin, has been given the status of preservative to be added in food items commercially. The US food and drug administration had given GRAS status to Nisin (7).

Nisin was first discovered in England in 1928 as a result of difficulty experienced during cheese making. Storage of milk had allowed contaminated organism to grow and produce inhibitor. The potential application of nisin in food preservation was demonstrated in 1951 and later its use as a food preservative were elaborated. Nisin have been concentrated as as Nisaplin which is used as preservative in milk, dairy products, canned foods, cured meats and other segments of fermentation industry. Nisin inhibits virtually all gram-positive bacteria in food. International acceptance of Nisin was given in 1969 (8).

Properties of bacteriocins

First discovered by Gratia in 1925, "principe V" was produced by one strain of E. coli against another culture of E. coli. The term "colicine" was coined by Gratia and Frederick(1946); "bacteriocine" was used by Jacob and others (1953) as a general term for highly specific antibacterial proteins. The term colicine now implies a bacteriocidal protein produced by varieties of E. coli and closely related Enterobacteriaceae (9).

Bacteriocins (as colicins) were originally defined as bacteriocidal proteins characterized by lethal biosynthesis, a very narrow range of activity, and adsorption to specific cell envelope receptors. Later, the recognized association of bacteriocin biosynthesis with plasmids was added to the description. The definition has since been modified to incorporate the properties of bacteriocins produced by gram-positive bacteria. Bacteriocins from grampositive bacteria commonly do not possess a specific receptor for adsorption although exceptions exist, are most frequently of lower molecular weight than colicins, have a broader range of target bacteria with different modes of release and cell transport, and possess leader sequences cleaved during maturation. Today, bacteriocidal peptides or proteins produced by bacteria are typically referred to as bacteriocins. Usually, to demonstrate the proteinaceous nature of a newly characterized bacteriocin, sensitivity to proteolytic enzymes such as trypsin, α-chymotrypsin, and pepsin is an expected demonstration (9).

Classification of bacteriocins

Bacteriocin have been grouped into four main distinict classes. On a sound scientific basis three defined classes of bacteriocins have been established: Class I, the lantibiotics; class II, the small heat stable non lantibiotics; and class III, large heat labile bacteriocins. A fourth class of bacteriocins is composed of an undefined mixture proteins, lipids and carbohydrates (10).

Class-I Lantibiotics characterized by the presence of unusual thioether amino acid which are generated through post translational modification.

Class-II Bacteriocin represent small (<10 kD) heat-stable hydrophobic, membrane active peptides.

Class-IIA Subclass II A represented by Listeria active peptides which contain the N terminally located consensus sequence YGNGCXV where X is any amino acid.

Class IIB Representing portion complexes that require two different peptides for activity.

Subclass-IIC Peptide whose externalizations into the growth medium of the producing bacterium is dependent on the general secretory pathway.

Class-III Bacteriocins belonging to class III consist of large (>30 kD) heat labile protein.

Class-IV Represent complex bacteriocin that contain essential lipid, carbohydrate moieties in addition to a protein compared.

Most of the bacteriocins from LAB are cationic, hydrophobic,or amphiphilic molecules composed of 20 to 60 amino acid residues. Lantibiotics (from lanthionine containing antibiotics) are small (<5 kDa) peptides containing the unusual amino acids lanthionine (Lan), α-methyllanthionine (MeLan), dehydroalanine, and dehydrobutyrine. These bacteriocins are grouped in class I. Class I is further subdivided into type A and type B lantibiotics according to chemical structures and antimicrobial activities. Type A lantibiotics are elongated peptides with a net positive charge that exert their activity through the formation of pores in bacterial membranes. Type B lantibiotics are smaller globular peptides and have a negative or no net charge; antimicrobial activity is related to the inhibition of specific enzymes.

Small (<10 kDa), heat-stable, non-lanthionine-containing peptides are contained in class II. The largest group of bacteriocins in this classification system, these peptides are divided into 3 sub-groups. Class IIa includes pediocin-like peptides having an N-terminal consensus sequence -Tyr-Gly-Asn-Gly-Val-Xaa-Cys. This subgroup has attracted much of the attention due to their anti-Listeria activity. Class IIb contains bacteriocins requiring two different peptides for activity, and class IIc contains the remaining peptides of the class, including sec-dependent secreted bacteriocins.

The class III bacteriocins are not as well characterized. This group houses large (>30 kDa) heat-labile proteins that are of lesser interest to food scientists. A fourth class consisting of complex bacteriocins that require carbohydrate or lipid moieties for activity has also been suggested; however, bacteriocins in this class have not been characterized adequately at the biochemical level to the extent that the definition of this class requires additional descriptive information (8).

Table 1—Examples of bacteriocins.

BACTERIOCINS	PRODUCER
Class I-type A lantibiotics	
Nisin	Lactococcuslactis
lactocin S	Lactobacillus
epidermin	Staphylococcus epidermidis
gallidermin	Staphylococcus gallinarum
lacticin 481	L. lactis
Class I-type B lantibiotics	
Mersacidin	Bacillus subtilis
Cinnamycin	Streptomyces cinnamoneus
ancovenin	Streptomyces ssp.
Duramycin	S. cinnamoneus
Actagardin	Actinoplanes ssp.

Class IIa	
pediocin PA-1/AcH	Pediococcusacidilactici
sakacin A	L. sake
sakacin P	L. sake
leucocin A-UAL 187	Leuconostocgelidum
mesentericinY105	Leuconostocmesenteroides
enterocin A	Enterococcus faecium
divercinV41	Carnobacteriumdivergens
lactococcin MMFII	L. lactis
Class IIb	
lactococcin G	L.lactis
lactococcin M	L. lactis
lactacin F	Lactobacillus
plantaricin A	Lactobacillus plantarum
plantaricin S	L. plantarum
plantaricinEF	L. plantarum
plantaricinJK	L. plantarum
Class IIc	
acidocin B	Lactobacillus acidophilus
carnobacteriocinA	Carnobacteriumpiscicola
divergicin A	C. divergens
enterocin P	E. faecium
enterocin B	E. faecium
Class III	
helveticin J	Lactobacillus heleveticus
helveticin V-1829	L. helveticus

Bacteriocins of gram positive bacterias versus gram negative

Bacteriocins of gram positive bacterias differ from gram-negative bacteriocins in two fundamental ways. First, bacteriocin production is not necessarily the lethal event it is for gram-negative bacteria. This critical difference is due to the transport mechanisms gram-positive bacteria encode to release bacteriocin toxin. Some have evolved a bacteriocin-specific transport system, whereas others employ the sec-dependent export pathway. In addition, the gram-positive bacteria have evolved bacteriocin-specific regulation, whereas bacteriocins of gram-negative bacteria rely solely on host regulatory networks. Gram-positive bacteriocins in general and lantibiotics in particular require many more genes for their production than do gram-negative bacteriocins. Several gram-positive bacteriocins, including nisin, are located on transposons (13).

Bacteriocins produced by Gram-positive and Gram-negative bacteria differ into several ecological and evolutionary aspects. In Gram-positive bacteria, the biosynthesis of bacteriocins is self-regulated and bacteriocin production is not a lethal event. In addition, the spectrum of antimicrobial activity is broader than the peptides from gram-negative species and bacteriocin release is controlled by specific regulatory mechanisms. In Gram-positive bacteria the gene clusters for bacteriocin production are generally organized in the chromosome and include genes encoding the pre-peptide, and proteins responsible for post-translational modifications, regulation, immunity and transport across the cytoplasmic membrane. In contrast, Gram-negative bacteria are often killed by bacteriocin production, the release of the peptide is controlled by common regulatory mechanisms, and specific genes encoding proteins responsible for cell lysis are common (23).

Bacteriocins versus antibiotics

Although therapeutic antibiotics are prohibited for use in foods, the utilization of antagonistic additives with preservative or antimicrobial properties has since become a trademark approach in food safety and preservation. In foods and beverages, addition of antimicrobial compounds to processed products has become a traditional weapon in the food preservation arsenal. Comprising a subgroup within the far larger body of commercial food preservatives are the bacteriocins. Bacteriocins are produced by bacteria and possess antibiotic properties, but bacteriocins are normally not termed antibiotics in order to avoid confusion and concern with therapeutic antibiotics that can potentially illicit allergic reactions in humans. Bacteriocins differ from most therapeutic antibiotics in being proteinaceous and generally possessing a narrow specificity of action against strains of the same or closely related species. Bacteriocins are ribosomally synthesized polypeptides possessing bacteriocidal activity that are rapidly digested by proteases in the human digestive tract (11)

Bacteriocins are often confused in the literature with antibiotics (12). This would limit their use in food applications from a legal standpoint. In some countries, it is critical to make the distinction between bacteriocins and antibiotics. The main differences between bacteriocins and antibiotics are summarized in Table. Bacteriocins, which are clearly distinguishable from clinical antibiotics, should be safely and effectively used to control the growth of target pathogens in foods. Bacteriocins can be differentiated from antibiotics on the basis of synthesis, mode of action, antimicrobial spectrum, toxicity and resistance mechanisms. Recognizing that bacteriocins are different from antibiotics, the term biological food preservatives since bacteriocins, unlike antibiotics, are not used for medicinal purposes.

Table 2: Bacteriocins vs. antibiotics.

Characteristic	Bacteriocins	Antibiotics
Application	Food	Clinical
Synthesis	Ribosomal	Secondary metabolite
Activity	Narrow spectrum	Varying spectrum
Host cell immunity	Yes	No
Mechanism of target cell resistance or tolerance	Usually adaptation affecting cell membrane composition	Usually a genetically transferrable Determinant affecting different sites depending the mode of action
Interaction requirements	Sometimes docking molecules	Specific targets
Mode of action	Mostly pore formation, but in a few cases possibly cell wall biosynthesis	Cell membrane or intracellular targets
Toxicity or side effects	None known	Yes

While all classes of bacteriocins are ribosomally synthesized, only Class I is post-translationally modified to produce the active form. Different from bacteriocins, antibiotics are generally considered secondary metabolites. Antibiotics are not ribosomally synthesized. Although several antibiotics, such as vancomycin, are composed of amino acids, they are enzymatically synthesized. (13)

Mode of action

The bactericidal action of bacteriocin occurs at the cytoplasmic membrane of target cells In general, bacteriocins act by binding to the charged headgroups of phospholipids of the membrane or to a proteinaceous receptor, inserting into the membrane, and forming pores in the cytoplasmic membrane. This results in a depletion of the proton motive force, which interferes with cellular biosynthesis and causes cell death. In general, bacteriocins act mainly by pore formation in target cell membranes, or by inhibiting cell wall synthesis or enzyme activities in the cytosol (RNAse or DNAse) (14)

The anionic lipids of the cytoplasmic membrane are the primary receptors for bacteriocins of LAB for initiation of pore formation. Conductivity and stability of pores induced by lantibiotics may be heightened by docking molecules (lipid II, the peptidoglycan precursor), while in the case of class II bacteriocins, receptors in the target membrane apparently act to determine specificity. Class I bacteriocins may induce pore formation according to a wedge-like model, and class II bacteriocins may function by creating barrelstave-like pores or a carpet mechanism whereby peptides orient parallel to the membrane surface and interfere with membrane structure.

Most of the bacteriocins are bactericidal with some exceptions LeucocinA UAL 187 being bacteriostatic. Inhibitory activity of bacteriocin producing strains are mostly confined to gram-positive bacteria. Bacteriocins are bactericidal to sensitive cells. Death of indicator occur rapidly at a very low concentration. Sensitivity of gram-positive and sensitivity of gram-negative bacteria towards bacteriocins has been demonstrated on the basis of cell wall composition. It has been observed that gram-negative bacteria become sensitive towards bacteriocin action if it is destabilized by physical or chemical stresses. Gram-negative bacteria possess an additional layer so called outer layer which is composed of phospholipids proteins and lipopolysacharides and this layer is impermeable to most molecules. Presence of porin in this layer allow free diffusion of molecular with a molecular mass below 600Da. The smallest bacteriocin produced LAB are approximately 3 kD and too large to reach their target cytoplasmic membrane. It has been proposed that Nisin act on cytoplasmic membrane of gram-positive bacteria to cause lesions. Following nisin treatment whole or intact sensitive cells and membrane vesicles exhibit efflux of amino acid and cations. Lose of these substances depletes proton motive force, which ultimately interferes with cellular biosynthesis. These events result in collapse of membrane potential and ultimately cause cellular death. Similarly, other bacteriocins such as Lactococin A, Pediocin JD, etc. have also been reported to cause dissipation of the membrane potential and increase in membrane permeability to ions leading to collapse of proton motive force (15).

Activity Spectra and Properties of Class I and Class IIa Bacteriocins

Most of the class I bacteriocins have a fairly broad inhibitory spectrum. They not only inhibit closely related bacteria, such as species from the genera Enterococcus, Lactobacillus, Lactococcus, Leuconostoc, Pediococcus, and Streptococcus, but also inhibit many less closely related gram-positive bacteria, such as L. monocytogenes, Staphylococcus aureus, Bacillus cereus, and Clostridium botulinum. Several bacteriocins in this class, such as nisin and thermophilin 13, prevent outgrowth of spores of B. cereus and C. botulinum. Interestingly, acidocin J1132 has a very narrow inhibitory spectrum and sensitive strains are limited to members of the genus Lactobacillus, while at the other extreme, plantaricin LP84 (produced by Lactobacillus plantarumNCIM 2084) has demonstrated antagonism against E. coli (15)

Compared to class I bacteriocins, most class IIa Bacteriocins have comparatively narrow activity spectra and only inhibit closely related gram-positive bacteria. In general, members of the genera Enterococcus, Lactobacillus, Pediococcus are sensitive to class IIa bacteriocins, and members of the genus Lactococcus are resistant. Some class IIa bacteriocins, such as pediocin PA-1, have fairly broad inhibitory spectra and can inhibit some less closely related gram-positive bacteria, such as S. aureus and vegetative cells of Clostridium spp. and Bacillus spp. Some class IIa bacteriocins, such as mundticin from Enterococcus mundtii, even prevent the outgrowth of spores of C. botulinum.

It might seem that bacteriocins with broader activity spectra would always be preferable for use in food preservation, but under certain circumstances bacteriocins with narrower inhibitory spectra may prove more desirable. For example, sakacin P, which has limited activity against LAB but is nearly as effective as pediocin PA-1 against Listeria, might find application in LAB fermentation products that are prone to contamination by L. monocytogenes (16).

Bacteriocin immunity

The immunity of the cell synthesizing the bacteriocin to its product is a phenomenon that distinguishes bacteriocins from antibiotics. Genes coding for immunity proteins are in close genetic proximity to other bacteriocin structural and processing genes (17).

Bacteriocin producing cells are protected against their own bacteriocin as long as they are present in the –inactive – precursor form. The leader sequences of bacteriocins that direct their excretion often consist of 18 to 30 amino acid residues. The final step of bacteriocin biosynthesis is secretion of the precursor. Often after secretion, helix breaking residues at the positions 2 and/or 1 of the cleavage site may serve as re-cognition sites for the leader peptidase that cleaves this bond to release the mature and active peptide in the medium. Bacteriocin secretion systems are thought to contribute to self protection or immunity of the bacteriocin producing strain (18).

Use of bacteriocin as food bio-preservatives

Recent interest to isolate bacteriocin producing strains is due to its effectiveness against food spoilage/pathogenic bacteria and also due to its proteinaceous nature which made it safer for human consumption. It is assumed to be degraded by protease in gastrointestinal track (5). Digestive enzymes rapidly inactivate bacteriocin and consequently it cannot alter bacterial microflora in the intestinal track. Many regulatory agencies have advocated the use of hurdle concept, i.e. combining several physical and chemical methods for preservation in sub optimal levels to control microbial growth. Pediocin when combined with low dose irradiation showed a greater inhibitory effect on growth of L. mesenteroides (19).

The following requirements should be fulfilled by any bio-preservative to be used commercially.

1. The bio-preservative to be used should not be toxic.
2. It should be accepted by recognized authorities.
3. It should be economical to the industries using it.
4. The product in which the bio-preservative is being used should not be affected by it, i.e. bio-preservative should not show any deleterious effect toward the organoleptic properties of that product.
5. When used at relatively low concentrations it should show effect.
6. The bio-preservative should be sufficiently stable if being stored.
7. It should not have any medicinal use.

Bacteriocins fulfill all the above requirements and hence are gaining popularity in the food industry day-by-day.

Bacteriocins and Food Preservation

The only bacteriocins currently employed in food preservation are those produced by LAB used in the production of fermented foods. Because LAB have been used for centuries to ferment foods, they enjoy GRAS (generally regarded as safe) status by the U.S. Food and Drug Administration (FDA). This permits their use in fermented foods without additional regulatory approval. Nisin was the first bacteriocin to be isolated and approved for use in foods, specifically to prevent the outgrowth of Clostridium botulinum spores in cheese spreads in England. By 1988, the FDA had approved its use as a biopreservative for a narrow range of foods, including pasteurized egg products. Today, nisin is accepted as a safe food preservative by over 45 countries, and it is the most widely used commercial bacteriocin and it remains the only bacteriocin that may be added to U.S. foods. Over the past decade the recurrence of listeriosis outbreaks, combined with the natural resistance of the causative agent, Listeria monocytogenes, to traditional food preservation methods such as its ability to grow at near-freezing temperatures has focused the attention of bacteriocin researchers on this organism. This attention has resulted in the isolation of a large number of class IIa bacteriocins, all of which are highly active against L. monocytogenes (23).

The next wave of development of bacteriocins as food preservatives is at hand. Bacteriocins have been discovered in cured meats, milk and cheese, spoiled salad dressing, and soybean paste. Luchansky and colleagues have developed a gelatin form of pediocin, a class IIa bacteriocin made by lactic acid–producing bacteria, that protects hot dogs from Listeria contamination. His team has also added a strain of pediocin-producing bacteria to sausage and found a reduction of Listeria numbers to be fewer than one ten-thousandth the original number in untreated sausage. Equally compelling, active pediocin was found in the sausage after two months of refrigeration. At the University of Melbourne in Australia, Barrie Davidson has been targeting Listeria with piscicolin, a bacteriocin from yet another lactic acid–producing bacterium. Piscicolin has already been patented and it will soon be ready for use in meat products and as a rinse for salad greens or chicken parts.

A natural concern about using bacteriocins for the preservation of food is the selection of resistant strains. Studies in LAB have shown that resistance carries a significant fitness cost, with resistant strains having a slower growth rate than their sensitive ancestor. Treatment with a combination of bacteriocins, for instance nisin and a class IIa bacteriocin, would theoretically reduce the incidence of resistance. There is currently conflicting evidence as to whether resistance to one class of LAB bacteriocins can result in cross-resistance to another class.

In recent years, concerns about the safety and quality of foods have increased the attention given to the discovery and development of new methods of preservation of foods. The source of bacteriocins for application to foods can be either a purified compound, a crude bacterial fermentate, or the bacteriocin-producing organism (20).

Different methods of applications of bacteriocin in different food items

Bacteriocines have been incorporated into different food items by following various techniques such as (21):

1. Direct soaking food items into bacteriocin solution.
2. Using polyethylene-based plastic fillms and edible cellulosic films.
3. Adsorption of bacteriocin on different surfaces such as polyethylene, ethylene vinyl acetate, polypropylene, polyanile, polyester, acrylic, polyvinyl chloride and salanized silica etc.
4. Antimicrobial casing containing bacteriocin preparation also aid preservative effect.
5. Bacteriocin producing LAB cultures can be used in hurdle technology strategies to reduce food borne disease.

Potential and Challenges of bacteriocins application

The use of bacteriocins has the potential to allow the food industry to better predict the storage lives of its products and provides an additional barrier to the growth of food-borne pathogens. However, There are some challenges ahead for expanded use of bacteriocins in foods. Regulatory restrictions and requirements will slow the widespread application to foods. A better understanding of the development of bacteriocin-resistant pathogens in foods and strategies to mitigate their emergence need to be developed. A lack of consumer acceptance of genetically modified organisms in foods could be a barrier to the expanded possibilities of genetically improved starter cultures for the production of bacteriocins. However, as consumers continue to demand products that are minimally processed and preserved, the use of bacteriocins may become more common as a means of "naturally" preserving foods.

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