



Review Paper

BACTERIOCINS: A NEW TREND IN ANTIMICROBIAL FOOD PACKAGING

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Abstract: *Bacteriocins are proteaceous toxin produced by bacteria to inhibit the growth of similar or closely related bacteria. Among lactic acid bacteria bacteriocins are produced by Streptococcus, E.coli, Pediococcus, Pseudomonas, Carnobacterium, Enterococcus, and Staphylococcus. Bacteriocins are classified by various methods such as method of killing, genetic, molecular weight and method of production.*

As there are many more applications of bacteriocins in various industries such as food, pharmaceutical, cattle and poultry feed industry etc. besides this one more important application of bacteriocins is, it can be used as antimicrobial film for packaging of food items. The antimicrobial film is prepared by incorporation of antimicrobial substances into the extruder when the film or the co – extruded film is produced.

Keywords: *Lactic acid bacteria, Bacteriocins, Antimicrobial food Packaging.*

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INTRODUCTION

What are bacteriocins?

Historically the term “Bacteriocin” was applied to antibiotic like compounds with specificity primarily restricted to bacterial strains. This can be thought of as microbial “murder” of ones relative, but their specificity and chemical composition served to distinguish them from so called classical antibiotics. (Reeves, 1979).

To go back to the first bacteriocin descriptions amounts to studying the first works concerning bacterial antagonism. Such bacterial antagonism was described by the pioneers of microbiology during the last decades of the 19th century. At that time, the molecular basis of bacterial inhibition was abstruse, so it was difficult to distinguish antagonism due to bacteriocin from that provoked by other compounds such as antibiotics, organic acids, or hydrogen peroxide, except on the basis of their spectrum of activity, usually narrower than that of other ones. (Desriac, 2010).

The historical definition of bacteriocins applies to the most widely studied group. Gratia (1925), observed the production of a highly specific antibiotic substances by one strain of *E.coli* which was active against another strain of the same species. The name colicin was given to such substances produced by *E. coli* and other members of the family Enterobactriaceae, (Anantnarayan, 1978, Desriac, 2010). This bacteriocin definition is based on the properties of the colicins, that is to say, a lethal biosynthesis, a very narrow spectrum of activity limited to the same species as the producer bacteria and a receptor mediated mechanism of action. (Jumriangrit, 2004).

In those days, during fifties and sixties, the bacteriocins world was mainly made up of bacteriocins from Gram negative bacteria. And three genera of Gram positive bacteria were studied for bacteriocin production. *Bacillus Sp.*, *Listeria Sp.*, *Staphylococcus Sp.* Specific antagonism between related bacteria was reported in the 1920s. But term colicin was not coined until 1946 and the more general term bacteriocin in 1953 (Konisky, 1978, Reeves, 1972).

In 21 century, the bacteriocin is defined as, ribosomally synthesized biologically active proteins or protein complexes having bactericidal or bacteriostatic mode of action and displaying antimicrobial activity spectrum towards Gram positive bacteria and particularly



towards closely related species of producers strain. (Sansit, 2004, Yamazaki 2005, Jumriangrit 2004, Beasley, 2004).

Naming and classification of bacteriocins:

A Uniform system of naming bacteriocin does not exist. (Konisky, 1978). Names are generally derived from the producing genus or species. For example Colicin from *E. coli*, Pyocin from the *Pseudomonas pyocynea*, Megacins from *Bacillus megaterium* and Diphthericins from *Corynebacterium diphtheria*.

Classification of bacteriocin:

According to the classification procedure proposed by Klaenhammer and modified by Nes *et al.*, (Klaenhammer, 1993) bacteriocins produced by lactic acid bacteria (LAB) are classified in to four main groups or classes. These classes of the bacteriocin are shown in the Table 1. Out of which lantibiotics or Class I being the most documented and industrially exploited group. Classification of bacteriocin from Gram Positive bacteria (Jumriangrit, 2004).

Table :1. Classes of bacteriocins based on their molecular weight.

Class	Molecular Mass	Characteristics and subclass
I	< 5 KDa	Ribosomally produced peptides that undergo extensive post – translational modification, small peptides containing lanthionin and beta methyl lanthionine.
II	< 10 KDa	Low molecular weight, heat stable peptides. Formed exclusively by unmodified amino acids, ribosomally synthesized as inactive prepeptides that get activated by post translational cleavage of the N terminal leader peptide. II a: anti – listerial single peptides that contain YGNGV amino acids motif near their N termini. II b: two peptide bacteriocins. IIc: thiol – activated peptides.
III	> 30KDa	High molecular weight, heat labile proteins.
IV		Complex bacteriocins carrying lipid or carbohydrate moieties.

Most bacteriocins that have been studied in lactic acid bacteria are in class I or class II.

Class I – Lantibiotics:

Bacteriocins in this fact that they are heat resistant and therefore they are of more interest for use in food preservation than heat labile class III bacteriocins. Class I had low molecular weight of less than 5 KDa. (Jumriangrit, 2004).



Lantibiotics are bacteriocins characterized by presence of lanthionine type thioester bond between certain side chains of amino acids residues of the protein. It is proposed that, lantibiotic synthesis and export involved three distinct phases:

1. Intracellular post translational modification of specific amino acids.
2. Transport of fully modified peptides across the cytoplasmic membrane.
3. Proteolytic cleavage of the leader peptides releasing the mature and active peptide into the surrounding medium. (MC Auliffe, 2001).

According to their chemical structure and their antimicrobial activities the lantibiotics are subdivided into type A and B lantibiotics. (Sahl, 1998). Type A lantibiotics, which are elongated cationic peptides up to 34 residues in length that show similarities in arrangement of their LAN bridges. These bacteriocins peptide primarily kill target organism by disrupting their membrane integrity. Examples are of type A lantibiotics: Nisin, Epidermin, Pep 5, and Lactocon 5. In this group nisin is most studied.

Type B lantibiotics, which are globular peptides and have a low net positive charge, up to 19 residues in length, and their antimicrobial activity is related to disruption enzyme function of the target organism. For example inhibit enzymes involved in cell wall biosynthesis. Examples of bacteriocins in this type B lantibiotics are Mersacidin, Actagardin and Cinnamycin.

Class II: Non lantibiotics:

These bacteriocins are the most abundant and thoroughly studied in all of four subclasses. They are a heterogeneous group of small (<10 KDa), heat stable peptides. They are ribosomally synthesized as inactive prepeptides that are modified by post translational cleavage of the N terminal leader peptide generally at a double glycine to release mature cationic peptides that are amphipathic and thermostable. However class II bacteriocins can be divided in three groups. (Eijsink, 2002, Gnnahar, 2000, MC Auliffe, 2001),).

Subclass IIa bacteriocins, the largest subgroup which is active to *Listeria*. These peptides are characterized by the presence of the conserved motif. The YGNVG box, which is usually located within the N terminus of the mature peptide.

Subclass IIb bacteriocins, which are two peptides bacteriocins that require both peptide for optimum inhibitory activity. These two structural genes of active two peptide are in same operon.



Subclass IIc peptides are referred to the thiol activated peptides, which require a reduced cysteine for activity.

Class III: Large heat labile bacteriocins.

Class III bacteriocins are high molecular weight and heat labile proteins. They have been isolated from members of genus *Lactobacillus*. (Klaenhammer, 1993). This class may therefore include bacteriolytic extracellular enzyme (hemolysin and muramidases) that may mimic the physiological activities of bacteriocins. Examples of class III bacteriocins are Caseicin 80, Helveticin J, and Helveticin LP 27.

BACTERIOCINS PRODUCED BY LACTIC ACID BACTERIA:

Lactic acid bacteria which include the genera *Lactococcus*, *Streptococcus*, *Lactobacillus*, *Pediococcus*, *Leuconostoc*, *Enterococcus*, *Carnobacterium* and *Propionibacterium*. Thus lactobacilli are divided into homofermentative and heterofermentative.

Lactic acid bacteria in particular produce a wide variety of antimicrobial substances including organic acids, hydrogen peroxide, diacetyl, secretory reaction products and bacteriocins which have potential to inhibit a variety of other microorganisms. The extensive interest in bacteriocins of lactic acid bacteria and the potential commercial value of these antimicrobials in food preservation and other biological applications have been well documented. (Devuyst, 1994, Ross, 1999).

The bacteriocins produced by lactic acid bacteria are shown in Table 2. (Klaenhammer, 1993).

Table: 2 General overview of the bacteriocin and their producer strains.

Bacteriocin	Producer strain	Class	Subclass	Molecular mass	Mode of action
Carnocin U 149	<i>Carnobacterium piscicola</i> U 149	I	II c	4.6 KDa	Pore forming
Lacticin 481	<i>Lactococcus lactis</i> 481	I		2.9 KDa	Pore forming
Lactococcin	<i>Lactococcus lactis</i> ADr 185 L030	I	II d	2.3 KDa	Pore forming
Nisin	<i>Lactococcus lactis</i> subsp. <i>Lactis</i>	I	A type	3.5 KDa	Pore forming
Salivaricin	<i>Streptococcus salivarius</i>	I		2.3 KDa	Pore forming
Microcin B 17	<i>Escherichia coli</i>	I		3.1 KDa	Intracellular enzymes
Pediocin	<i>Pediococcus</i>	I	II a	4.6 KDa	Pore forming



Colicin	<i>Escherichia coli</i>		Group A	40.80KDa	Nuclease / pore forming
Caranobacteriocin	<i>Caranobacterium iscolica LV 17 B</i>	II		4.5KDa	
Pyocin	<i>Pseudomonas aeruginosa</i>			270KDa	Pore forming
Helveticin	<i>Lactobacillus helveticus</i>	II		37.5KDa	To be defined
Enterolysin	<i>Enterococcus faecalis</i>	III		34.5 KDa	Peptidoglycan hydrolysis
Lysostaphin	<i>Staphylococcus aureus</i>	III		25 KDa	Peptidoglycan hydrolysis

MODE OF ACTION:

The antibiotic activity of bacteriocin from Gram positive bacteria is based on interaction with bacterial membrane. Some of bacteriocins elaborated amphiphilic property generalized membrane disruption by pore formation. Lactic acid bacteria produce several types of pore forming peptides. Most of bacteriocins produced from lactic acid bacteria are bactericidal peptides which act primarily by creating pores in the membrane of their target cells. Although the formation of pore is a general feature. The size, stability and conductivity of these pores differ considerably from bacteriocins to bacteriocins. The formation of poration complexes, causing an ionic imbalance and leakage of inorganic phosphate. These mechanisms rely upon stabilizing interactions between membrane phospholipids and the cationic residues of the peptides allowing the insertion of hydrophobic regions into the outer leaflet of the membrane. One associated with the membrane surface a number of the ordered bacteriocins could potentially aggregate.

The bacteriocin complex can in principle completely span the membrane thereby forming a transient pore. (McCormick, 1998, Nettles, 1993). In which there is dissipation of proton motive force (PMF), which involves the partial or total dissipation of either or both the transmembrane potential and a pH gradient.

Anyway most bacteriocin interacts with anionic lipids that are abundantly present in the membrane of Gram Positive Bacteria. Theses anionic lipids may enhance the conductivity and stability of antibiotic pores by as docking molecule or may acts as receptors in class II bacteriocins.(Hechard, 2002, Moll, 1999).



ANTIMICROBIAL FOOD PACKAGING:

Antimicrobial packaging is one of many applications of active packaging. Antimicrobial packaging is the packaging system that is able to kill or inhibit spoilage and pathogenic microorganisms that are contaminating foods. The new antimicrobial function can be achieved by adding antimicrobial agents in the packaging system or using antimicrobial polymer that satisfy conventional packaging materials have to extend the lag phase and reduce growth rate of microorganisms to prolong the self-life and maintain food quality and safety.(Han,2000).

Antimicrobial agents may be incorporated into the packaging materials initially and migrate into the food through diffusion and partitioning. (Han, 2000).besides diffusion and equilibrated sorption, some antimicrobial or fungicide or active moieties such as amine groups. In this case utilizes surface inhibition of microbial growth by immobilization of the non-food grade antimicrobial substances without diffusion mass transfer.

The incorporation of antimicrobial substances into a food packaging system can take several approaches. One is to put the antimicrobial into the film by adding it in the extruder when the film or the co-extruded film is produced.

MODELING OF AN ANTIMICROBIAL FILM:

According to Han (2000), several factors must be taken into account in the design or modeling of antimicrobial film.

1. Chemical nature of film, casting process condition and residual antimicrobial activity.
2. Characteristics of antimicrobial substances and foods.
3. Storage temperature.
4. Mass transfer coefficient.
5. Physical properties of packaging material.

Besides the microbial characteristics, the characteristics of antimicrobial function of the antimicrobial agent are also important to understand the efficacy as well as the limits of the activity.

INTRODUCTION OF THE “ACTIVE” FOOD PACKAGING:

Approved on 27th October 2004 by the European parliament and the council of the European Union Rules 1935/2004, concerning materials and objects destined to come into contact with food products. It also defines the role of EFSA, the European Food Safety



Authority set up by the so-called “food law”. Moreover, it forces that materials and objects that have not yet come into contact with food product at the moment of their entering the market must be marked with the wording “suitable for contact with food”. Finally it requires that all materials and objects that come into contact with food must be traceable in order to facilitate the control and with drawl of defective product.

The main principle are that active packaging must protect the food and not misled the consumer, therefore solutions that hide the state of deterioration of a food will never be authorized. Another much desired intervention concerns the “Declaration of conformity” foreseen under 1935/2004.

APPLICATION OF BACTERIOCIN IN FOOD PRESERVATION AND PACKAGING FILM:

As preservatives:

Application of bacteriocin in food preservation and hygiene. The principle physical, chemical, enzymatic and microbiological reactions responsible for food deterioration are well known. Various preservation techniques to avoid different forms of spoilage and food poisoning, including reduction in temp, water activity and pH as well as addition of preservatives such as, antimycotic, inorganic and organic compounds are known to slow or prevent growth of microorganisms. (Ross, 1999).

Nisin, the bacteriocin produced by *Lactococcus lactis subsp. Lactis* has been applied as food preservatives in several countries. It has been used to control some food borne pathogens, especially some species of the genera *Aeromonas*, *Bacillus*, *Clostridium*, *Enterococcus*, *Listeria*, *Micrococcus*, and *Staphylococcus*. (Benkerroum, 2000).

The potential application of bacteriocins as consumer friendly.Biopreservatives either the form of protective culture or as additives is significant besides being less potentially toxic or carcinogenic than current antimicrobial agents, lactic acid bacteria and their byproducts have been shown to be more effective and flexible in several applications. In addition of that, functional properties in lactic acid bacteria improve preservatives effect and add flavor and taste. (Savadogo, 2004, Pal, 2004).

As Packaging Film:

Incorporation of bacteriocin into packaging films to control food spoilage and pathogenic organisms has been an area of active research for last decade. Antimicrobial packaging film



prevents microbial growth on food surface by direct contact of the package with the surface of foods, such as meat and cheese.

For this reason, for it to work, the antimicrobial packaging film must contact the surface of the food, so that bacteriocins can diffuse to the surface. The gradual release of bacteriocins from a packaging film to the food surface may have an advantage over dipping and spraying foods with bacteriocins. (Appendini, 1996).

Two methods have been commonly used to prepare packaging films with bacteriocins (Appendini, 1996). One is to incorporate bacteriocins directly into polymers. Example includes incorporation of nisin into biodegradable protein films. And other two packaging film – forming methods, heat press and casting. Were used to incorporate nisin into films made from soy protein and cornzein. Polyetelene based plastic film incorporated with nisin was used to vacuum pack bed carcasses. (Siragusa, 1999).

Another method to incorporate bacteriocin into packaging film is to coat or adsorb bacteriocins to polymer surface. Examples include nisin / methylcellulose coatings for polyetelene films and nisin coatings for poultry (Appending, 1996). It was demonstrated that, nisin absorbed onto salinized silica surfaces inhibited the growth of *Listeria monocytogenes*. Nisin films were exposed to medium containing *Listeria monocytogenes* and the contacting surfaces were evaluated at 4 hours intervals for 12 hours. Cells on surface that had been in contact with a high concentration of nisin (40000 IU/ml) exhibited no signs of growth and many displayed evidence of cellular deterioration.

The efficacy of bacteriocins coating on the inhibition of pathogens has also been demonstrated in other studies. For example, coating of pediocin onto cellulose casing and plastic bags has been found to completely inhibit growth on inoculated *Listeria monocytogenes* in meat and poultry through 12 week storage at 4 degree centigrade. (Ming, 1997).

Although shelf life was extended in food products as population of food spoilage organisms were reduced, the primary thrust was towards control of specific anticipated pathogens in the product.

Among the emerging conservation technologies is the use of natural additives such as egg white lysozyme and bacteriocins of lactic acid bacteria to control the growth or survival of undesirable microorganisms. Nisin has been employed for over 50 years and is currently



approved in more than 60 countries for use in cheese, liquid egg products, canned vegetables, diverse pasteurized dairy and salad dressing. It is only purified antibiotic peptide that has been licensed for utilization as food preservatives by the US food and Drug Administration. (Jumriangrit, 2004).

AREAS OF RESEARCH IN ANTIMICROBIAL FOOD PACKAGING WITH BACTERIOCIN:

1. Increasing the spectrum of bacteriocin with respect to antimicrobial activity.
2. increasing the efficacy of currently used bacteriocin
3. Searching of novel bacteriocin producer strains.

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