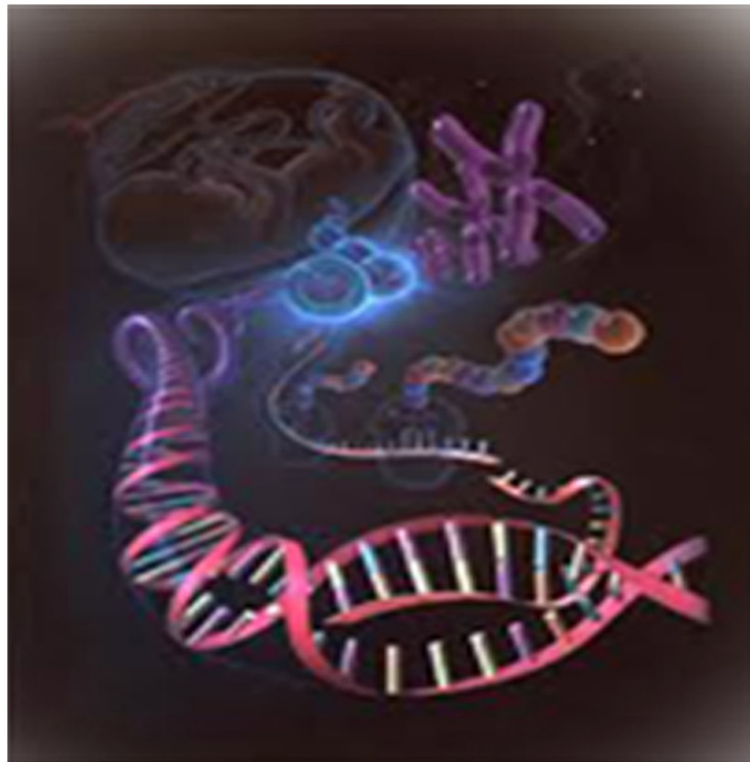




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Review Article

BACTERIOCINS: MODERN CONCEPT IN AGRO-PRODUCT AND BY-PRODUCT PRESERVATION

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One of the major concern in food and agro industries is the contamination of food by pathogens, which are frequent cause of food borne diseases and deterioration. A recent trend in food preservation was directed towards employing bacteriocins as preservative to improve food safety and shelf life of Agro by-products. Bacteriocins are antimicrobial proteins produced by bacteria which kill or inhibit the growth of other bacterial. These antimicrobial proteins are produced by lactic acid bacteria. They are classified into three, and examples of some species that produces bacteriocin are *lactococcus lactis*, *lactobacillus acidophilus*, *leuconostoc mesenteroides*, etc. Several strategies have been explained on its applications in food which include inoculation of a food product with lactic acid bacteria that manufacture bacteriocin and the use of an ingredient in food processing that has been previously fermented with bacteriocin-producing bacteria. The mechanism of bacteriocins action is inhibition of target cells by forming pores in the membrane, resulting in leakage of cellular material which causes the cell membrane of the spoilage bacteria to lysis. Bacteriocins especially nisin is safe for human consumption at acceptable intake of 2.9 mg/day. They are used in the preservations of Agro by-product like cheese, milk, meat, etc. Therefore if properly harnessed from microorganisms, they will help solve the problem of Agro products and by-products storage and preservation in order to boost shelf life and free from pathogens. Since they are not toxic and does not change the organoleptic of the food product but destroys spoilage microorganism in food, bacteriocins should gain popularity in food industries and agro industries.

Keywords: Bacteriocin, Contamination, Preservation, By-product, Food safety

INTRODUCTION

One of the concerns in food industry is the contamination by pathogens, which are frequent cause of food borne diseases. Over the past decade, recurrent outbreaks of diarrhea,

combined with the natural resistance of the causative agents, contributed to its status as hazard (Parada *et al.*, 2007). A recent trend in food preservation is directed towards employing minimal chemical additives and thermal

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treatment, but with assured microbial safety and shelf life stability to comply with consumers' demands for natural and healthy food (Touch *et al.*, 2009). Such demands have led to the exploitation of non-thermal preservation methods to control undesirable microorganisms while retaining both nutritional and sensory properties of the food. The use of natural preservatives is viewed as an appropriate alternative because they are readily available in plants, animals, insects, and microorganisms where they evolve as part of their hosts' defense mechanisms against microbial invasion (Parada *et al.*, 2007)

Biopreservation is defined as the use of non-pathogenic microorganisms and/or their metabolites to improve microbiological safety and extend the shelf life of foods (De Martinis *et al.*, 2001). Among the exploited biopreservative, the antimicrobials peptide, called bacteriocin, which is produced by Lactic Acid Bacteria (LAB) has been found to offer several desirable properties such as heat stability, wider pH tolerance, relatively broad spectrum activity, bactericidal and inactivation by digestive proteases, which make them suitable for food preservation. Bacteriocin are ribosomally synthesized antimicrobial peptide that are active against other bacteria, either in the same species (narrow spectrum), or across genera (broad spectrum) (Klaenhammer, 1988). Several lactic acid bacteria bacteriocins have been isolated, characterized and found to possess great potential in the preservation of food and food products. Nisin is approved for use in over 40 countries and has been in use as a food preservative for over 50 years (Cleveland *et al.*, 2001). Though nisin is currently the only bacteriocin approved for use in the United States, many bacteriocins produced by members of the LAB have potential application in food products.

BACTERIOICIN

Nature of Bacteriocin

Historically, it was observed that *Escherichia coli* cells produced in liquid medium a substance stable to temperature oscillations and also possessors of inhibitory activity on the growth of other taxonomically similar microorganisms. It was, probably, the initial step for studies involving bacteriocins (Jack *et al.*, 1995). Originally, these substances were termed "colicins", because most of the studies observed their synthesis by *E. coli* cells. After verifying that the synthesis of these molecules was common among other bacteria, the common name "bacteriocin" to designate them (Jacob *et al.*, 1953). Currently, Bacteriocins have been detected in all major lineages of Eubacteria and Archaeobacteria (Torrebranca *et al.*, 1995). Bacteriocins are antibacterial proteins or peptide produced by bacteria that kill or inhibit the growth of other bacteria. They are synthesized ribosomally, extracellularly released and are active against other bacteria, either in the same species (narrow spectrum), or across genera (broad spectrum) (Klaenhammer, 1988 and Cleveland *et al.*, 2001). The producer organisms are immune to their own bacteriocin(s), a property that is mediated by specific immunity proteins (Cotter *et al.*, 2005). Both Gram-negative and Gram-positive bacteria produce small heat stable bacteriocins, but so far, they are found less frequently in Gram-negative bacteria (Cotter *et al.*, 2005). The Gram-positive group LAB has been documented to produce a large variety of bacteriocins (Cleveland *et al.*, 2001). Most of the bacteriocin-producing LABs are isolated from endogenous fermented food, making them a potential biopreservative group of microorganisms. Bacteriocins differ from

most therapeutic antibiotics in being proteinaceous agents that are rapidly digested by proteases in the human digestive tract. They are ribosomally synthesized peptides, and this fact creates the possibility of improving their characteristics to enhance their activity and spectra of action (Saavedra *et al.*, 2004).

CLASSIFICATION OF BACTERIOCIN

There is a wide number of bacteriocins produced by different LAB (Table 1), and they can be classified according to their biochemical and genetic characteristics (Klaenhammer *et al.*, 1993; Nes *et al.*, 1996; González-Martínez *et al.*, 2003).

Class I- Lantibiotics: small (< 5 kDa) heat-stable peptides acting on membrane structures. They are extensively modified after translation, resulting in the formation of characteristic thioether aminoacids lanthionine and methylanthionine. These arise via a two-step process, originated from post-translational modifications: firstly, gene-encoded serine and threonine are subjected to enzymatic dehydration to give rise to dehydroalanine and dehydrobutyrine, respectively (Sahl and Bierbaum, 1998). A very well-known example of this group is nisin. The lantibiotic bacteriocins were initially divided into two subclasses based on structural similarities.

Subclass Ia: included relatively elongated, flexible and positively charged peptides; they generally act by forming pores in the cytoplasmic membranes of sensitive target species. The prototypic lantibiotic nisin is a member of this group.

Subclass Ib: peptides are characteristically

globular, more rigid in structure and are either negatively charged or have no net charge. They exert their action by interfering with essential enzymatic reactions of sensitive bacteria (Deegan *et al.*, 2006).

Class II. – Non-Lantibiotics: bacteriocins of variable molecular weight, but usually small (<10 kDa), heat-stable, containing regular amino-acids. This group was divided into three subgroups:

Class IIa: peptides active against *Listeria*, the characteristic represents are pediocin PA-1 (Venema *et al.*, 1997 and sakacin P).

Class IIb: formed by a complex of two distinct peptides. These peptides have little or no activity and it appears to be no sequence similarities between complementary peptides. In this group are lactococcin G and plantaricins EF e JK.

Class IIc: Small peptides, heat-stable, which are transported by leader-peptides. In this subclass are found only the bacteriocins divergicin A and acidocin B.

Class III. – Large peptides, with molecular weight over 30 kDa. In this class are helveticins J (Joerger and Klaenhammer, 1986) and V (Vaughan *et al.*, 1992), acidofilicin A and lactacins A and B. Most of the low molecular weight bacteriocins are highly cationic at pH 7.0, and this seems to be a unifying feature of both the lantibiotics and non-lantibiotics (Cintas *et al.*, 2001)

BACTERIOCINS AND ANTIBIOTICS

Bacteriocins are often confused in the literature with antibiotics, they differ from antibiotics on the basis of their synthesis, mode of action, toxicity and resistance mechanism.

Table 1: Bacteriocins of Lactic Acid Bacteria and Their Spectra of Activities

Producing species	Bacteriocin	Spectrum of action
<i>Lactococcus lactis</i> subsp. <i>Lactis</i>	Nisin Lacticin 3147	Gram-positive bacteria <i>Clostridium</i> sp <i>Listeria monocytogenes</i> <i>Staphylococcus aureus</i> <i>Streptococcus dysgalactiae</i> <i>Enterococcus faecalis</i> <i>Propionibacterium acne</i> <i>Streptococcus mutans</i>
<i>Lactococcus lactis</i> subsp. <i>Cremonis</i>	Lactococcin B	<i>Lactobacillus</i>
<i>Lactobacillus acidophilus</i>	Acidocin CH5 Lactacin F	Gram-positive bacteria <i>Lactobacillus fermentum</i> <i>Enterococcus faecalis</i> <i>Lactobacillus delbrueckii</i> <i>Lactobacillus helveticus</i>
Lactacin B	<i>Lactobacillus delbrueckii</i>	<i>Lactobacillus helveticus</i> <i>Lactobacillus bulgaricus</i> .
<i>Lactococcus lactis</i> .	<i>Lactobacillus amylovorus</i>	Lactobin A <i>Lactobacillus acidophilus</i> <i>Lactobacillus delbrueckii</i>
<i>Lactobacillus casei</i>	Lactocin 705	<i>Listeria monocytogenes</i> <i>Lactobacillus plantarum</i>
<i>Leuconostoc gelidum</i>	Leucocin A	<i>Lactobacillus</i> <i>Enterococcus faecalis</i> <i>Listeria monocytogenes</i>
<i>Leuconostoc mesenteroides</i>	Mesentericin Y105	<i>Enterococcus faecalis</i> <i>Listeria monocytogenes</i>
<i>Pediococcus acidilactici</i>	Pediocin F	Gram-positive bacteria
	Pediocin PA-1	<i>Listeria monocytogenes</i>
	Pediocin AcH	Gram-positive and Gram-negative bacteria under stressing situations
<i>Pediococcus pentosaceus</i>	Pediocin A	<i>Lactobacillus</i> <i>Lactococcus</i> <i>Leuconostoc</i> <i>Pediococcus</i> <i>Staphylococcus</i> <i>Enterococcus</i> <i>Listeria</i> <i>Clostridium</i>
<i>Enterococcus faecium</i>	Enterocin A	<i>Listeria monocytogenes</i> <i>Pediococcus</i>
<i>Lactobacillus sake</i>	Lactocin S Sakacin P	<i>Lactobacillus</i> <i>Leuconostoc</i> <i>Pediococcus</i>

Source: Parada et al., (2007)

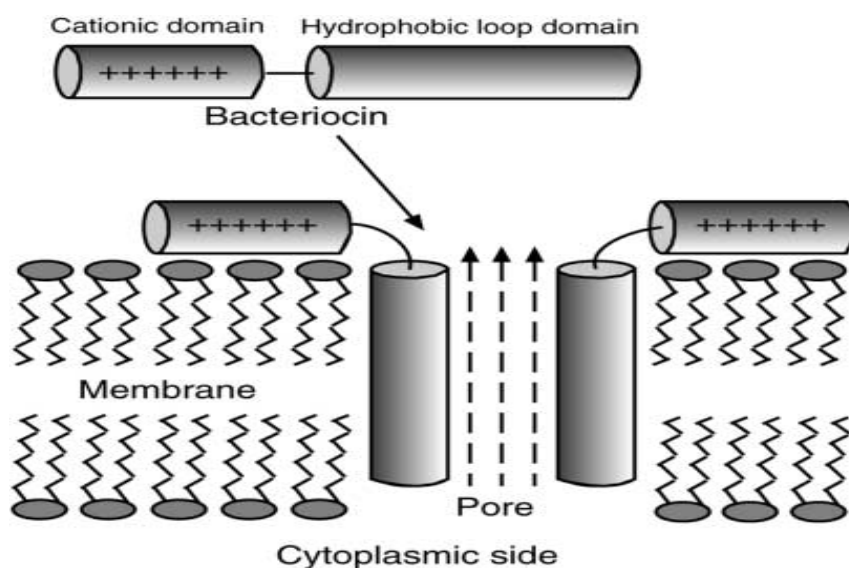
Mechanism of Bacteriocin Action

Bacteriocins are usually effective against Gram-positive microorganisms. Bacteriocins of lactic acid bacteria may be inefficient to inhibit Gram-negative organisms because the outer membrane hinders the site for bacteriocin action, which is the cell membrane. Different mechanisms of action have been proposed for bacteriocins: alteration of enzymatic activity, inhibition of spore germination and inactivation of anionic carriers through the formation of selective and non-selective pores (Parada *et al.*, 2007). Although the mode of activity of bacteriocins can differ, the cell envelop is commonly their target. The cell wall of gram-positive bacteria allows passage of relatively large molecules, so that there is unlikely to be a requirement for bacteriocin receptors analogous to those in the outer membranes of gram-negative cells. Anionic cell surface polymers like teichoic acid and lipoteichoic acid may be important in the initial interaction of cationic bacteriocins of Gram-positive bacteria (Jack *et al.*, 1995).

Bacteriocins, particularly lantibiotics, inhibit target cells by forming pores in the membrane, depleting the transmembrane potential and/ or the pH gradient, resulting in the leakage of cellular materials. Bacteriocins are positively charged molecules with hydrophobic patches. Electrostatic interactions with negatively charged phosphate groups on target cell membranes are thought to contribute to the initial binding with the target membrane (Cleveland *et al.*, 2001).

It is likely that the hydrophobic portion inserts into the membrane, forming pores (Figure 1). There is debate over the types of pores formed by nisin, with most groups favoring the “barrel-stave” or “wedge” models. In the “barrel-stave” model, each nisin molecule orients itself perpendicular to the membrane, forming an ion channel that spans the membrane, while in the “wedge” model, a critical number of nisin molecules associate with the membrane, they insert concurrently, forming a wedge (Ojcius and Young, 1991; Driessen *et al.*, 1995). More recent

Figure 1: Mechanism of Bacteriocin Action (Breukink et al., 1999)



studies demonstrate the complexity of bacteriocin activity, where nisin must bind to lipid II, a docking molecule, on the susceptible cell membrane in order to kill (Breukink *et al.*, 1999). Instead of pore formation occurring indiscriminately, it appears that “docking molecules” on the target cell membrane facilitate the interaction with the bacteriocin, thereby increasing the effectiveness of the bacteriocin. This mechanism has been clearly demonstrated for nisin and mersacidin, which both use lipid II, a peptidoglycan precursor, as a docking molecule (Breukink *et al.*, 1999; Brotz *et al.*, 1998a, and Cleveland *et al.*, 2001)

Requirement Fulfilled by Bacteriocin

The following requirements was fulfilled by Bacteriocin to be used commercially

- Bacteriocins are not toxic.
- It's economical to the industries using it.
- It does not show any deleterious effect toward the organoleptic properties of that products.
- Bacteriocins show effect when used at relatively low concentration.
- It's sufficiently stable if being stored. (Parada *et al.*, 2007)

Acute, subchronic and chronic toxicity studies, as well as reproduction, sensitization, *in vitro* and cross-resistance studies showed bacteriocin especially nisin is safe for human consumption.

Bacteriocins have been consumed for centuries as products of lactic acid bacteria. The approval of bacteriocin was based on published and unpublished data regarding its safety.

Application of Bacteriocin in Food and Agricultural By-Products

Several strategies have been explained on the applications of bacteriocin in food. The commonly used methods include:

1. The use of bacteriocin producing culture in the production of the food.
2. The use of purified bacteriocin, in liquid or powdered form as additive.
3. The combination of bacteriocin and other preservative (chemical or physical) agent. That is the hurdle technology.

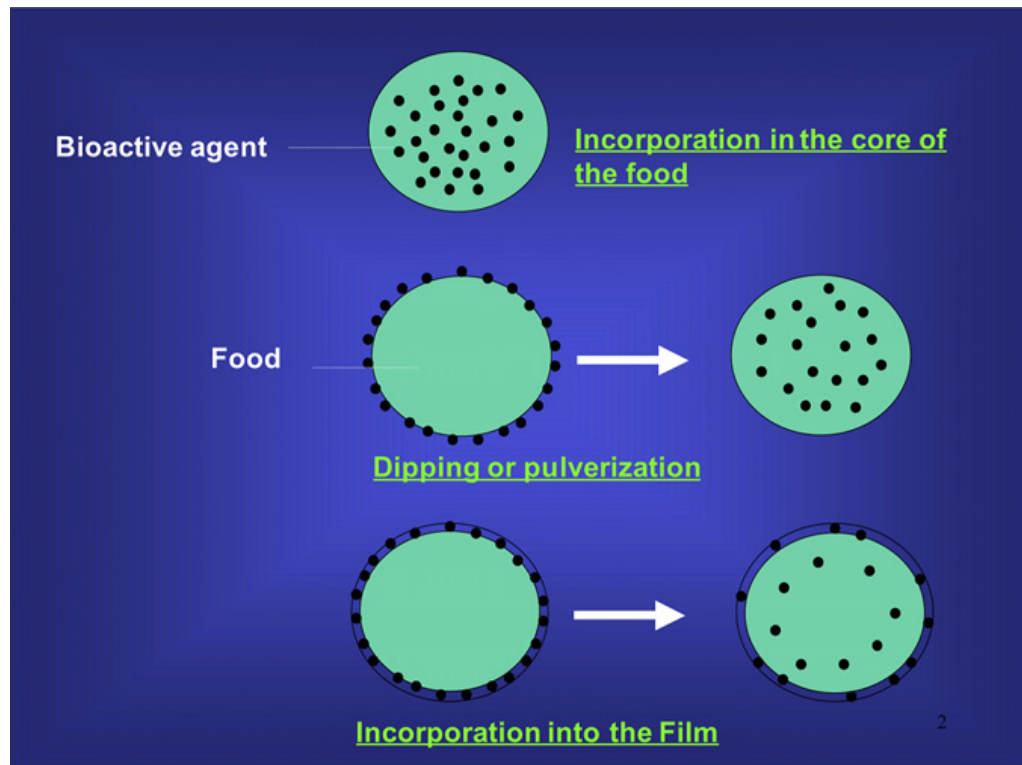
According to Coma *et al.* (2001), other methods of incorporating bacteriocins in food and agro by-products is included in Figure 2 below:

The intensive study on the applications of bacteriocins in the preservation of food such as diary, meat and poultry, fish and sea food, vegetable and drinks was recently reported by (Galvez *et al.*, 2008).

Bacteriocins in Dairy Products

The main bacterial pathogens of concern in the dairy industry are those able to survive and multiply in the raw materials as well as in certain types of cheese, such as *Listeria monocytogenes*, *Staphylococcus aureus*, *Escherichia coli*, and *Salmonella* spp. (Galvez *et al.*, 2008). Spoilage of semi-hard and hard cheeses (e.g., Kasseri, Emmental, Gruyere, Grana, Edam and Gouda) due to gas formation by *Clostridium tyrobutyricum* also of great economical concern. Therefore, most investigations concerning application of bacteriocins in dairy foods have focused in the use of bacteriocin in preventing and controlling the spoilage of dairy products (Galvez *et al.*, 2008). Nisin (in the commercial form Nisaplin) has been tested extensively in dairy foods. One of the earliest applications was to prevent gas blowing in cheese caused by *C. tyrobutyricum* Nisin has found many other applications in the dairy industry, especially in processed cheeses

Figure 2: Different Incorporation Modes of Bacteriocin in Food Products and Consequences (Coma et al., 2001)



and cheese products (e.g., block cheese, softwhite cheeses, slices, spreads, sauces, dips) to prevent proliferation of surviving endospore formers, mainly the gas-producing clostridia and *Clostridium botulinum*, as well as other post process contaminating bacteria such as *L. monocytogenes* (Galvez et al., 2008). It is also used in other pasteurized dairy products, such as chilled desserts, flavored milk, clotted cream, or canned evaporated milks. In heat-treated cream, growth of *Bacillus cereus* during storage was completely inhibited by low concentrations of nisin (Nissen et al., 2001). In sliced cheese, immobilized nisin in a polyethylene/polyamide packaging was shown to reduce the population of LAB, *Listeria innocua* and *S. aureus* (Galvez et al., 2008). However, the use of nisin in cheese

fermentation may interfere with growth of starter cultures, and have detrimental effects on acidification and/or aroma formation. To protect starters from the detrimental action of nisin during cheese production (and also to enhance nisin stability), nisin Z encapsulated into liposomes was successfully tested and found to inhibit listeria in cheddar cheese (Galvez et al., 2008).

Bacteriocins in Meat

Meat and meat products provide the necessary nutrients for growth of a wide variety of microorganisms, depending on the storage conditions. Under refrigeration, oxygen availability will allow the growth of aerobic gram-negative bacteria (especially *Pseudomonas*), where as LAB (mainly, *Carnobacterium*, *Lactobacillus*, and

Leuconostoc) predominate under anaerobic conditions (Galvez *et al.*, 2008). Cooked meat products are also at risk for microbial growth due to their generally low salt content (2% on average), high pH (around 6.0) and water activity (above 0.945), and lack of competing microbiota. Vacuum or modified atmosphere packaging and refrigeration storage are often used to prolong shelf-life. During storage, changes in the gaseous phase occur, and CO₂-tolerant slow-growing bacteria proliferate. These are represented mainly by *Lactobacilli* (predominantly, *Lactobacillus sakei* and *Lactobacillus curvatus*, as well as *Leuconostoc carnosum*, *Leuconostoc gasicomitatum*, *Leuconostoc mesenteroides*, *Weissella* spp., and *Carnobacterium* spp.) (Galvez *et al.*, 2008). These LAB represent the major spoilage bacteria, causing defects such as souring, off-flavor, discoloration, gas production, slime formation, and pH decrease (Aznar and Chenoll, 2006; Chenoll *et al.*, 2007). *Brochothrix thermosphacta* may also grow under certain conditions (depending mainly on film permeability, residual oxygen, and pH), producing a cheese-like odor (Galvez *et al.*, 2008). The processing environment may also facilitate proliferation of bacterial pathogens, especially *L. monocytogenes* (Thevenot *et al.*, 2006).

Moreover, because cooked, ready-to-eat meat products offer no protection against pathogenic or spoilage organisms on post processing contamination, growth of pathogenic bacteria (mainly, *L. monocytogenes*) and spoilage bacteria are currently the major concerns (Galvez *et al.*, 2008). Therefore, the bacteriocins and their producer bacterial strains may have several potential applications in the meat industry. Several reports have described the effects of added

bacteriocin preparations on inhibition of spoilage and pathogenic bacteria in fresh meats (Galvez *et al.*, 2008). In beef meat, addition of pediocin PA-1 retarded growth of meat spoilage gram-positive bacteria (Kalchayanand, 1990) and reduced the *L. monocytogenes* population (Nielsen *et al.*, 1990). Similar studies confirmed the anti listerial protection of pediocins in raw meats (Galvez *et al.*, 2008).

Bacteriocin in Poultry Product

Another field of application of bacteriocin is the preservation of egg and egg product (Galvez *et al.*, 2008). The commercial use of liquid whole egg requires processing in order to prolong its shelf-life and to inactivate foodborne pathogens. The fact that *Listeria* species have been isolated from commercially broken raw liquid whole egg and that, contrary to salmonella, conventional minimal egg pasteurization processes do not grant a complete inactivation of this pathogen has moved researchers to test bacteriocins as antilisterial hurdles in this food system (Schuman and Sheldon, 2003). Addition of nisin to pasteurized liquid whole egg reduced the viable counts of *L. monocytogenes*, increased the product refrigerated shelf-life, and protected the liquid egg from growth of *L. monocytogenes* and *B. cereus* during storage (Galvez *et al.*, 2008). Both nisin and pediocin Pa1/Ach acted synergistically with heat treatments against *L. monocytogenes*, and nisin addition increased the heat sensitivity of *Salmonella enteritidis* PT4 in liquid whole egg and in egg white during pasteurization (Galvez *et al.*, 2008).

Bacteriocins have been tested in liquid egg in combination with PEFs and HHP treatments. Exposure of *L. innocua* to nisin after PEF treatment at low temperature showed an additive

to synergistic effect, depending on the bacteriocin concentration and the electric field intensity and number of pulses (Galvez *et al.*, 2008). Nisin in combination with HHP treatment markedly reduced viable cell counts of *E. coli* and *L. innocua*, and the two microorganisms were not detectable after 1 month of refrigeration storage of the treated liquid whole egg (Ponce *et al.*, 1998).

Bacteriocins in Fish and Sea Foods

The microbial ecology of sea food products has been reviewed (Galvez *et al.*, 2008). Different microbial groups may be selected during sea food storage, depending on the preservation conditions. In fish products preserved by the addition of low NaCl levels, slight acidification, and chill storage in vacuum packs (e.g., cold-smoked fish), the predominant microflora is composed by LAB (mainly, *Lactobacillus* and *Carnobacterium*), with some gram-negative bacteria (*Photobacterium phosphoreum* and psychrotrophic *Enterobacteriaceae*) (Galvez *et al.*, 2008). Inadequate microbial control may result in product spoilage of vacuum-packaged cold-smoked sea food. *L. sakei*, *B. thermosphacta*, *Serratia liquefaciens*, and *P. phosphoreum* have been shown to be responsible for off odors in Cold-Smoked Salmon (CSS) (Stohr *et al.*, 2001). Increasing acidification or addition of preservatives still allows proliferation of lactobacilli and yeasts (e.g., marinated products), whereas application of mild heat treatments (e.g., sous vide products) may allow proliferation of surviving endospore formers (*Clostridium* or *Bacillus*) that may cause food spoilage and compromise the product safety (Lindstrom *et al.*, 2006). One of the main concerns in the seafood industry is contamination with *L. monocytogenes* (Galvez

et al., 2008). Among the seafoods of higher risk are the lightly preserved products (<6% water-phase salt, pH 5), such as smoked fish (hot and cold smoked), lightly salted (caviar, brined cooked shrimp, matjes herring), or marinated products (Huss *et al.*, 2000). Most of them have been (1) highly processed; (2) have an extended shelf-life at refrigeration temperatures; (3) can support growth of *L. monocytogenes*; and (4) are consumed without further cooking (Galvez *et al.*, 2008).

Smoked salmon and smoked rainbow trout are considered risk products for listeriosis (Galvez *et al.*, 2008). Contaminated cold-smoked rainbow trout, as well as cold- and hot-smoked products of rainbow trout, have been suspected as contaminating sources for listeriosis (Ericsson *et al.*, 1997; Miettinen *et al.*, 1999). Outbreaks of listeriosis associated with smoked mussels and raw oysters have also been reported and consequently, most of the research on application of bacteriocins in this field has focused on prevention and control of *L. monocytogenes* (Galvez *et al.*, 2008). Early research indicated a low effectiveness of nisin against *L. monocytogenes* in CSS because nisin delayed, but did not prevent growth of *L. monocytogenes* in vacuum packs. However, the antilisterial effect of nisin was clearly enhanced under a CO₂ atmosphere (Galvez *et al.*, 2008). In further works, nisin and ALTA combined with CO₂ atmosphere packaging were shown to be effective in controlling *L. monocytogenes* in smoked salmon (Szabo and Cahill, 1999). Nisin-coated plastic films had an increased effectiveness against strains of *L. monocytogenes* that had previously shown a higher nisin resistance on vacuum-packaged

CSS (Neetoo *et al.*, 2008). The immobilized nisin also inhibited the proliferation of the smoked salmon background microbiota, controlling its microbial spoilage. Application of a combination of nisin and Microgard reduced the total aerobic bacteria populations in fresh chilled salmon and reduced growth of *L. monocytogenes* in frozen-thawed salmon and increased its shelf-life (Galvez *et al.*, 2008). Nisin injected into smoked rainbow trout was able to inhibit growth of *L. monocytogenes* in the smoked fish, although it was more effective in combination with sodium lactate (Nykanen *et al.*, 2000).

Bacteriocins in Vegetable Foods and Drinks

The vegetable food and drink industries offer a wide variety of scenarios, depending on the raw materials, the processing conditions, and the final products. These vary from raw fruit and vegetables, ready-to eat vegetable foods, canned products, fermented vegetables, fruit juices and drinks, and beverages (Galvez *et al.*, 2008). One good example of the risks associated with vegetable foods is the consumption of seed sprouts, which has been ubiquitous for many centuries in oriental culture and has grown in global popularity during the past 30 years (Rosas and Escartin, 2000). Although consumption of seed sprouts has a "healthy" image, sprouts have been demonstrated to be the vehicle for transmission in a number of outbreaks of foodborne illness, involving several serotypes of *Salmonella*, *E. coli* O157, and *B. cereus* (Galvez *et al.*, 2008). Laboratory experiments have demonstrated that *L. monocytogenes* can proliferate rapidly on sprouting alfalfa seeds and on many other vegetable products (Galvez *et al.*, 2008). Several listeriosis outbreaks have been

associated with fresh produce, such as raw celery, tomatoes, and lettuce (Beuchat, 1996). Bacteriocins and bacteriocinogenic strains could be applied in the preservation of vegetable foods and drinks with several purposes. Competitive exclusion techniques, where nonpathogenic microorganisms are used to repress the growth of pathogenic bacteria during sprouting, have been suggested in several occasions. Isolation of naturally occurring microbes that produce antimicrobial substances against pathogens in fresh produce has been reported, and LAB strains have been shown to be effective in suppressing the growth of pathogens on ready-to-use vegetables (Galvez *et al.*, 2008).

Nisin-producing lactococci isolated from bean sprouts reduced the levels of *L. monocytogenes* after refrigeration storage (Galvez *et al.*, 2008). A mundticin-producing *Enterococcus mundtii* strain isolated from minimally processed vegetables inhibited the growth of *L. monocytogenes* on sterile vegetable medium but not on fresh mungbean sprouts (Bennik *et al.*, 1999). However, the produced mundticin was found to have potential as a biopreservative agent for modified atmosphere-stored mungbean sprouts when used in a washing step or a coating procedure (Bennik *et al.*, 1999). Application of exsitu-produced bacteriocins seems a reasonable alternative to avoid the problems of in situ bacteriocin production in vegetable foods. Because bacteriocins have not been reported to elicit adverse effects on vegetable cells or tissues, they could be applied for decontamination of fruits and vegetables, either alone or in combination with, but cautioned that regrowth of survivors during storage of the treated food stuffs should be taken into account (Galvez *et al.*, 2008).

CONCLUSION

In the light of the foregoing, bacteriocins as proteins synthesized by certain bacteria have been proved to be useful as preservative of agricultural products like milk, cheese and meat, etc. Since they are readily available, not toxic and does not change the organoleptics of food products; therefore, if properly harnessed from help solve the problems of agro products and by products storage and preservation in order to boost the shelf life of food product Bacteriocins unlike other carcinogenic chemical preservatives are important bacterial products that are non toxic to human cells or organs but can destroy spoilage microorganism in food. Since there is no traceable residual effects on humans that consume foods preserved with bacteriocins, human and food safety is guaranteed. Therefore, their use in food and agricultural industries should be encouraged

REFERENCES

1. Alves V F, Martinez R C R, Lavrador M A S and De Martinis E C P (2006), "Antilisterial activity of lactic acid bacteria inoculated on cooked ham", *Meat Sci.*, Vol. 74, pp. 623-627.
2. Antara N S, Sujaya I N, Yokota A, Asano K and Tomita F (2004), "Effects of indigenous starter cultures on the microbial and physicochemical characteristics of Urutan, a Balinese fermented sausage.*J.Biosci. Bioeng.*, Vol. 98, pp. 92-98.
3. Aznar R and Chenoll E (2006), "Intraspecific diversity of *Lactobacillus curvatus*, *Lactobacill plantarum*, *Lactobacillus sakei*, and *Leuconostoc mesenteroides* associated with vacuum packed meat product spoilage analyzed by randomly amplified polymorphic DNA PCR", *J.Food Prot.*, Vol. 69, pp. 2403-2410.
4. Azuma T, Bagenda D K, Yamamoto T, Hawai Y and Yamazaki K (2007), "Inhibition o *Listeria monocytogenes* by freeze-dried piscicocinCS526 fermentate in food}, *Lett.Appl. Microbiol.*, Vol. 44, pp. 138-144.
5. Bauer R, Nel H A and Dicks L M T (2003), "Pediocin PD-1 as a method to control growth of *Oenococcus oeni* in wine", *Am. J. Enol.Vitic.* , Vol. 5, No. 4, pp. 86-91.
6. Benkerroum N, Oubel H, Zahar M, Dlia S and Filali-Maltouf A (2000), "Isolation of a bacteriocin-producing *Lactococcus lactis* subsp.*lactis* and application to control *Listeria monocytogenes* in MoroccanJben", *J. Appl. Microbiol.*, Vol. 89, pp. 960-968.
7. Beuchat L R (1996), "Pathogenic microorganisms associated with fresh produce", *J. Food Prot.*, Vol. 59, pp. 204-216.
8. Breukink E, Wiedemann I, van Kraaij C, Kuipers O P, Sahl H and de Kruijff B (1999), "Use of cell wall precursor lipid II by a pore-forming peptide antibiotic", *Science*, Vol. 286, pp. 2361-2364.
9. Brotz H, Bierbaum G, Leopold K, Reynolds P E and Sahl H G (1998b), "The lantibiotic mersacidin inhibits peptidoglycan synthesisby targeting lipid II", *Antimicrob.Agents Chemother*, Vol. 42, pp. 154-160
10. Chenoll E, Macian M C, Elizaquivel P and Aznar R (2007), "Lactic acid bacteria associated with vacuum-packaged cooked-

- meat product spoilage: population analysis by rDNA based methods”, *J. Appl. Microbiol.* 102, pp. 498–508
11. Cleaveland J, Mantiville T J, Ness I F and Chiknids M L (2001), “Bacteriocins: safe antimicrobials for food preservation”, *International Journal of Food Microbiology*, Vol. 71, pp. 1-20.
 12. Coma V, Sebti I, Deschamps A and Pichavant H F (2001), “Antimicrobial edible packaging based on cellulosic ethers, fatty acids and nisin incorporation to inhibit *Listeria monocytogenes* and *Staphylococcus aureus*”, *Journal of Food Protection*, Vol. 64, pp. 470–475.
 13. Cotter P D, Hill C and Ross R P (2005), “Bacteriocins: developing innate immunity for food”, *Nature Rev. Microbiol.*, Vol. 3, pp. 777–788.
 14. Cutter C N and Siragusa G R (1996), “Reductions of *Listeria Brochothrix* and *thermosphacta* on beef following nisin spray treatments and vacuum packaging”, *Food Microbiol.*, Vol. 13, pp. 23-33.
 15. De Martinis E C P, Públio M R P, Santarosa P R and Freitas F Z (2001), “Antilisterial activity of lactic acid bacteria isolated from vacuum-packaged Brazilian meat and meat products”, *Braz. J. Microbiol.*, Vol. 32, pp. 32-37
 16. Deegan L H, Cotter P D, Hill C and Ross P (2006), “Bacteriocins: Biological tools for biopreservation and shelf-life extension”, *Int. Dairy J.*, Vol. 16, pp. 1058-1071.
 17. Dicks L M T, Mellett F D and Hoffman L C (2004), “Use of bacteriocin-producing starter cultures of *Lactobacillus plantarum* and *Lactobacillus curvatus* in production of ostrich meat salami”, *Meat Sci.*, Vol. 66, pp. 703–708.
 18. Driessen A J, van den Hooven H W, Kuiper W, van de K M, Sahl H G, Konings R N and Konings W N (1995), “Mechanistic studies of lantibiotic-induced permeabilization of phospholipid vesicles”, *Biochemistry*, Vol. 34, pp. 1606–1614
 19. Galvez A, Lopez R L and Abriouel H, Valdivia E and Omar N N (2008), “ Application of Bacteriocins in the Control of Food borne Pathogenic and Spoilage Bacteria”, *Crit. Rev. Biotechnol.*, Vol. 28, pp. 125–152.
 20. Garcya-Graells C, Masschalck B and Michiels C W (1999), “Inactivation of *Escherichia coli* n milk by high-hydrostatic-pressure treatment in combination with antimicrobial peptides”, *J. Food Prot.*, Vol. 62, pp. 1248–1254.
 21. González-Martínez B E, Gómez-Treviño M and Jiménez-Salas Z (2003), “Bacteriocin as deprobióticos”, *Rev. Salud Pública Nutrición*, Vol. 4, pp. 122-138.
 22. Grande M J, Lucas R, Valdivia E, Abriouel H, Maqueda M, Martíñez-Cañamero M, Ben Omar N and Gálvez A (2005), “Stability of enterocin AS-48 in fruit and vegetable juices”, *J. Food Prot.*, Vol. 68, pp. 2085–2094.
 23. Huss H H, Jørgensen L V and Fønnesbech Vogel B (2000), “Control options for *Listeria monocytogenes* in sea foods”, *Int. J. Food Microbiol.*, Vol. 62, pp. 267–274.
 24. Jacob F, Lwaff A, Siminovich A and

- Wallman E (1953), "Definition de quelques terms relatifs à la lysogenie", *Annales de Institut Pasteur.*, Vol. 84, pp. 222-224.
25. Joerger M C and Klaenhammer T R (1986), "Characterization and purification of helveticin J and evidence for a choromosomal determined bacteriocin produced by *Lactobacillus helveticus*", *J. Bacteriol.*, Vol. 167, pp. 439-446
26. Kalchayanand N (1990), "Extension of shelf-life of vacuum-packaged refrigerated fresh beef by bacteriocins of lactic acid bacteria", Ph.D. thesis, University of Wyoming, Laramie, WY.
27. Klaenhammer T R (1988), *Bacteriocin of lactic acid bacteria. Biochemie.*, Vol. 70, pp. 337-349
28. Klaenhammer T R (1993), "Genetics of bacteriocins produced by latic acid bacteria", *FEMS Microbiol. Rev.*, Vol. 12, pp. 39-45.
29. Lindstrom M, Kiviniemi K and Korkeala H (2006), "Hazard and control of group II (non proteolytic) *Clostridium botulinum* in modern food processing", *Int. J. Food Microbiol.* , Vol. 108, pp. 92-104.
30. Lindstrom M, Morkila M, Skytta E, Hyyti-Trees E, Lahteenmaki L, Hielm S, Ahvenainen R and Korkeala H (2001), "Inhibition of growth of non proteolytic *Clostridium botulinum* type B in sous vide cooked meat products is achieved by using thermal processing but not nisin", *J. Food Prot.*, Vol. 64, pp. 838-844.
31. Luders T, Birkemo G A, Fimland G, Nissen-Meyer J and Nes I F (2003), "Strong synergy between a eukaryotic antimicrobial peptide and bacteriocins from lactic acid bacteria", *Appl. Environ. Microbiol.*, Vol. 69, pp. 1797-1799.
32. Miettinen M K, Siitonen A, Heiskanen P, Haajanen H, Bjorkroth K J and Korkeala H J (1999), "Molecular epidemiology of an outbreak of febrile gastroenteritis caused by *Listeria monocytogenes* in cold-smoked rainbow trout", *J. Clin. Microbiol.*, Vol. 37, pp. 2358-2360.
33. Millette M, Le Tien C, Smoragiewicz W and Lacroix M (2007), "Inhibition of *Staphylococcus aureus* on beef by nisin-containing modified alginate films and beads", *Food Control* , Vol. 18, pp. 878-884.
34. Nes I F, Diep D B, Håvarstein L S, Brurberg M B, Eijsink V and Holo H (1996), "Biosynthesis of bacteriocins in lactic acid bacteria", *Antonie van Leeuwenhoek.*, Vol. 70, pp. 113-128
35. Nielsen J W, Dickson J S and Crouse J D (1990), "Use of a bacteriocin produced by *Pediococcus acidilactici* to inhibit *Listeria monocytogenes* associated with fresh meat", *Appl. Environ. Microbiol.*, Vol. 56, pp. 2142-2145.
36. Parada J L, Caron C R, Medeiros A B P and Soccol C R (2007), "Bacteriocins from Lactic Acid Bacteria, pp. Purification, Properties and use as Biopreservatives", *Brazilian Arch. of Biol. Technol.*, Vol. 50, No. 3, pp. 521-542.
37. Parente E and Hill C (1992), Inhibition of *Listeria* in buffer, broth, and milk by enterocin 1146, a bacteriocin produced by

- Enterococcus faecium*. *J. Food Prot.* 55, pp. 503–508.
38. Pepe O, Blaiotta G, Moschetti G, Greco T and Villani F (2003), "Rope-producing strains of *Bacillus* spp. from wheat bread and strategy for their control by lactic acid bacteria", *Appl. Environ. Microbiol.*, Vol. 69, pp. 2321–2329.
39. Ponce E, Pla R, Sendra E, Guamis B and Mor-Mur M (1998), "Combined effect of nisin and high hydrostatic pressure on destruction of *Listeria innocua* and *Escherichia coli* in liquid whole egg", *Int. J. Food Microbiol.*, Vol. 43, pp. 15–19.
40. Rosas C J and Escartin E F (2000), "Survival and growth of *Vibrio cholerae* O1, *Salmonella typhi* and *Escherichia coli* O157: H7 in alfalfa sprouts", *J. Food Sci.*, Vol. 65, pp. 162–165.
41. Saavedra L, Minahk C, Holgado A P, De R and Sesma F (2004), "Enhancement of the enterocin CRL35 activity by a synthetic peptide derived from the NH₂-terminal sequence", *Antimicrob. Agents Chemother.*, Vol. 48, pp. 2778-2781
42. Sahl H G and Bierbaum G (1998), "LANTIBIOTICS: Biosynthesis and biological activities of uniquely modified peptides from Gram-positive bacteria", *Annu. Rev. Microbiol.*, Vol. 52, pp. 41-79.
43. Schuman J D and Sheldon B W (2003), "Inhibition of *Listeria monocytogenes* in pH-adjusted pasteurized liquid whole egg", *J. Food Prot.*, Vol. 66, pp. 999–1006.
44. Solomakos N, Govaris A, Koidis P and Botsoglou N (2008), "The antimicrobial effect of thyme essential oil, nisin, and their combination against *Listeria monocytogenes* in minced beef during refrigerated storage", *Food Microbiol.*, Vol. 25, pp. 120–127.
45. Stohr V, Joffraud J J, Cardinal M and Leroi F (2001), "Spoilage potential and sensory profile associated with bacteria isolated from cold-smoked salmon", *Food Res. Int.*, Vol. 34, pp. 710-715
46. Thevenot D, Dernburg A and Vernozy-Rozand C (2006), "An updated review of *Listeria monocytogenes* in the pork meat industry and its products", *J. Appl. Microbiol.*, Vol. 101, pp. 7-17.
47. Torrebranca M, Meseguer I and Ventosa A (1995), "Bacteriocin biology", *Lett. Appl. Microbiol.*, Vol. 19, pp. 201-205
48. Touch V, Hayakawa S and Commins T (2009), "Natural antimicrobial proteins: a review of current challenges and solutions for food applications", *Asian J. Food Agro-Industry.*, Vol. 2, No. 1, pp. 1-16
49. Vaughan E E, Daly C and Fitzgerald G F (1992), "Identification and characterization of helveticin V- 1829, a bacteriocin produced by *Lactobacillus helveticus* 1829", *J. Appl. Bacteriol.*, Vol. 73, pp. 299-308.

