

Biopreservative: Bacteriocin its classification and applications in food

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Biopreservation is the method used for food preservation by using natural antimicrobials (biopreservative) and microbionthereby increasing the storage life of food. There is increasing demand by consumers for chemical free food due to their reported harmful effects. Bacteriocins are proteins or peptides having bactericidal activity that can control the growth of food spoilage bacteria. These substances are produced by different types of gram positive and gram negative micro-organisms with variable molecular weights, biochemical properties and mode of action. Most bacteriocins are relatively specific to a single bacterial strain or species is targeted without disrupting other microbial populations. Use of bacteriocin is one of the alternatives to overcome this problem. A number of applications of bacteriocin have been reported in the areas of pharmaceuticals, livestock, agriculture, etc. but due to the non-toxicity and non-residence properties, the biopreservative potential of bacteriocins to control food pathogenic micro-organism and increase the shelf life of food products is of major focus.

Key Words : Biopreservation, Classification, Application in food

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INTRODUCTION

Biopreservative is the use of natural or controlled microflora or antimicrobials as a way of preserving food extending its shelf life (Baust and Baust, 2006). Modern technologies implemented in food processing and microbiological food-safety standards have diminished, but not altogether eliminated, the likelihood of food-related illness and product spoilage in industrialized countries. The increasing consumption of precooked food, prone to temperature abuse, and the importation of

raw foods from developing countries are among the main causes of this situation (Ananou *et al.*, 2007). Food spoilage refers to the damage of the original nutritional value, texture, flavour of the food that eventually render food harmful to people and unsuitable to eat. (Nath *et al.*, 2014). Several bacterial pathogens including *Salmonella*, *Campylobacter jejuni*, *Escherichia coli* 0157:H7, *Listeria monocytogenes*, *Staphylococcus aureus* and *Clostridium botulinum* are found associated with such outbreaks. Food preservation is a continuous fight against micro-organisms spoiling the food or making it unsafe (Rasooli, 2007). In order to achieve improved food safety against such pathogens, food industry mostly relies on the application of chemical preservatives or more drastic physical treatments (e.g. high temperatures). These preservation techniques have many drawbacks which includes the proven toxicity of many of the

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commonest chemical preservatives (e.g. nitrites), the alteration of the organoleptic and nutritional properties of foods, and especially recent consumer demands for safe but minimally processed products without additives. To harmonize consumer demands with the necessary safety standards, traditional means of controlling microbial spoilage and safety hazards in foods are being replaced by combinations of innovative technologies that include biological antimicrobial systems such as lactic acid bacteria (LAB) and/or their metabolites. (Nath *et al.*, 2013; Hugas *et al.*, 2002 and Montville *et al.*, 1997).

Biopreservation :

Biopreservation, can be defined as the extension of shelf-life and food safety by the use of natural or controlled microbiota and/or their antimicrobial compounds (Stiles, 1996). Fermentation is one of the most common forms of food biopreservation, it is a process based on the growth of micro-organisms in foods, whether natural or added. These organisms mainly comprise lactic acid bacteria, which produce organic acids and other compounds that, in addition to antimicrobial properties, also confer unique flavours and textures to food products. Traditionally, a great number of foods have been protected against spoiling by natural processes of fermentation. Currently, fermented foods are increasing in popularity (60% of the diet in industrialized countries) (Holzapeel *et al.*, 1995). The starter cultures of fermented foods can be defined as preparations of one or several systems of micro-organisms that are applied to initiate the process of fermentation during food manufacture (Wigley *et al.*, 1999) fundamentally in the dairy industry and, currently, extended to other fermented foods such as meat, spirits, vegetable products, and juices. The bacteria used are selected depending on food type with the aim of positively affecting the physical, chemical, and biological composition of foods, providing attractive flavour properties for the consumer. To be used as starter cultures, micro-organisms must fulfil the standards of GRAS status (Generally Recognized As Safe by people and the scientific community) and at present no pathogenic nor toxigenic potential observed. In addition, use must be standardized and reproducible (Dass *et al.*, 1999). Food market globalization, the introduction of novel foods, new manufacturing processes and the growing demand for minimally processed, fresh-cut and ready-to-eat products may require a longer and more

complex food chain, increasing the risk of microbiological contamination. Thus, novel and complementary food preservation technologies that comply with these demands from “farm to fork” are continuously sought. Among alternative food preservation technologies, particular attention has been paid to biopreservation to extend the shelf-life and to enhance the hygienic quality, minimizing the impact on the nutritional and organoleptic properties of perishable food products. Biopreservation rationally exploits the antimicrobial potential of naturally occurring micro-organisms in food and/or their metabolites with a long history of safe use.

Lactic acid bacteria (LAB) :

LAB was realized as a group of biopreservative bacteria in the beginning of the 1900's based on their interaction with the food lab and plays an important role in food fermentation processes. LAB is widely used in food preservation, microbial stability and production of aroma compounds (Narayanapillai Udhayashree *et al.*, 2012). LAB are characterized as Gram-positive cocci or rods, non-aerobic (microaerophilic) but aerotolerant, able to ferment carbohydrates for energy and lactic acid production. The metabolic pathway from glucose may be homofermentative or heterofermentative. Lactic acid bacteria are also able to produce small organic substances that contribute with aroma and give specific organoleptic attributes to the products (Caplice and Fitzgerald, 1999). LAB have attractive physiological properties and technological applications such as resistance to bacteriophages (Wigley *et al.*, 1999), proteolytic activity, lactose and citrate fermentation, production of polysaccharides, high resistance to freezing and lyophilization, capacity for adhesion and colonization of the digestive mucosa, and production of antimicrobial substances.

These micro-organisms are found in milk, meat, fermented products, as well as in fermented vegetables and beverages inhibiting the growth of pathogenic and deteriorating micro-organisms, maintaining the nutritive quality and improving the shelf-life of foods. They have also been used as flavour and texture developers. Lactic acid bacteria include various major genera: *Lactobacillus*, *Lactococcus*, *Carnobacterium*, *Enterococcus*, *Lactosphaera*, *Leuconostoc*, *Melissococcus*, *Oenococcus*, *Pediococcus*, *Streptococcus*,

Tetragenococcus, *Vagococcus* and *Weissella*. Other genera are: *Aerococcus*, *Microbacterium*, *Propionibacterium* and *Bifidobacterium* (Carr *et al.*, 2002). The most common species are *Lactobacillus acidophilus*, *L. plantarum*, *L. casei*, *L. caseirhamnosus*, *L. delbrueckii bulgaricus*, *L. fermentum*, *L. reuteri*, *Lactococcus lactis*, *Lactococcus lactis cremoris*, *Bifidobacterium bifidum*, *B. infantis*, *B. adolescentis*, *B. longum*, *B. breve*, *Enterococcus faecalis*, *Enterococcus faecium* (Garrity, 1984), and some strains are recognized as probiotics (Fuller, 1989; Parada *et al.*, 2003). LAB are usually known as safe (GRAS), and have an important role in the preservation of foods and fermented products. They can be used as natural competitive microbiota or as specific starter cultures under controlled conditions (Cintas *et al.*, 2001). Some of these bacteria produce antagonistic substances, called bacteriocins, which in small amounts are very active against pathogens (Moreno *et al.*, 2000).

LAB bacteriocins :

Bacteriocins are proteinaceous toxins produced by bacteria to inhibit the growth of similar or closely related bacterial strain(s) (Kathir, 2005). The antimicrobial ribosomally synthesized peptides produced by bacteria, including members of the LAB, are called bacteriocins. Bacteriocins are peptides or complex proteins biologically active with antimicrobial action against other bacteria, principally closely related species. They are produced by bacteria and are normally not termed antibiotics in order to avoid confusion and concern with therapeutic antibiotics, which can potentially illicit allergic reactions in humans and other medical problems (Deraz *et al.*, 2005). Bacteriocins differ from most therapeutic antibiotics in being proteinaceous agents that are rapidly digested by proteases in the human digestive tract. Since, bacteriocins are ribosomally synthesized; there exists a possibility of improving their characteristics to enhance their intensity and spectra of action (Nath *et al.*, 2013; Saavedra *et al.*, 2004). Colicine was the first bacteriocin, discovered in 1925 by André Gratia and his workgroup (Jacob *et al.*, 1953).

Attractive characteristics of the LAB bacteriocins which make them suitable candidates for use as food preservatives are as follows, (Nath *et al.*, 2014):

- Protein nature, inactivation by proteolytic enzymes of gastrointestinal tract

- Non-toxic to laboratory animals tested and generally non-immunogenic
- Inactive against eukaryotic cells
- Generally thermoresistant (can maintain antimicrobial activity after pasteurization and sterilization)
 - Broad bactericidal activity affecting most of the Gram-positive bacteria and some, damaged, Gram-negative bacteria including various pathogens such as *L. monocytogenes*, *Bacillus cereus*, *S. aureus*, and *Salmonella*
 - Genetic determinants generally located in plasmid, which facilitates genetic manipulation to increase the variety of natural peptide analogues with desirable characteristics.

Range of activity :

They form a heterogeneous group considering the antimicrobial spectrum, producing species, molecular weight, stability, physical chemical properties and mode of action of bacteriocins. There is the classic type, which has a spectrum of activity only against homologous species, and a second type, less common, which shows action against a wide range of Gram-positive micro-organisms. One example of this second type is nisin, which is produced by certain strains of *Lactococcus lactis* subsp. *lactis* (De Vuyst and Vandamme, 1992; Rodriguez *et al.*, 2005; Moreno *et al.*, 2000). Other is pediocin, produced by *Pedococcus pentosaceus* (Moreno *et al.*, 2000).

Nisin, produced by *L. lactis* subsp. *lactis*, which is active against Gram-negative bacteria, but only when used at high concentrations or when the target cells have been pre-treated with EDTA (Stevens *et al.*, 1991). Nisin is effective against food-borne pathogens such as *L. monocytogenes* and many other Gram-positive spoilage micro-organisms (Delves-Broughton, 1999 and Thomas and Delves-Broughton, 2001). Nisin is listed in Spain as E-234, and may also be cited as nisin preservative or natural preservative. In addition to the nisin, several authors have outlined issues involved in the approval of new bacteriocins for food use (Fields, 1996).

Bacteriocins are not frequently active against Gram-negative bacteria. The outer membrane of this class of bacteria acts as a permeability barrier for the cell. It is responsible for preventing molecules such as antibiotics, detergents and dyes from reaching the cytoplasmic

membrane (Stevens *et al.*, 1991). Some studies have already reported bacteriocin activity against this group of bacteria. Examples are plantaricin 35d, produced by *Lactobacillus plantarum* and active against *Aeromonashydrophila* (Messi *et al.*, 2001); bacteriocin ST151BR, produced by *Lactobacillus pentosus* ST151BR (Torodov and Dicks, 2004) and a bacteriocin produced by *Lactobacillus paracasei* subsp. *paracasei* active against *Escherichia coli* (Caridi, 2002); thermophylin, produced by *Streptococcus thermophilus* active against *E. coli*, *Yersinia pseudotuberculosis* and *Yersinia enterocolitica* among the gram-negative species and against several *Bacillus* species, *Listeria monocytogenes* and *Salmonella typhimurium* among the gram-positives (Ivanova *et al.*, 1998). Torodov and Dicks, 2005 reported that bacteriocins ST28MS and ST26MS, produced by *Lactobacillus plantarum* isolated from molasses inhibited the growth of *Escherichia coli* and *Acinetobacter baumannii* along with some Gram positive bacteria. Lade *et al.* (2006) have isolated two *Lactobacillus* species (*L. plantarum* and *L. lactis*) from vegetable waste that produced a bacteriocin which inhibited the growth of *E. coli*.

Classification of LAB bacteriocins :

Jeevaratnam *et al.*, 2005 have classified the LAB bacteriocins into four general classes of antimicrobial peptides or proteins (bacteriocins) and have characterized.

- Lantibiotics
- Small (≤ 13 kDa) hydrophobic heat stable peptides
- Large (~30 kDa) heat-labile proteins
- Complex proteins that require additional carbohydrates or lipids moieties to attain antimicrobial activity

Class I (Lantibiotics) :

Lantibiotics are a family of membrane active peptides that contain the unusual thio-ether amino acids lanthionine and β -methyl lanthionine as well as other modified amino acids such as gene-encoded serine and threonine are subjected to enzymatic dehydration to give rise to dehydroalanine and dehydrobutyrine, respectively (Jung, 1991). They are small (< 5 kDa) heat-stable peptides acting on membrane structures (Sahl and

Bierbaum, 1998). A very well-known example of this group is nisin (Broadbent *et al.*, 1989). Their distinguishing feature is the presence of post-translationally modified amino acid residues. The best example in this class is nisin produced by *Lactococcus lactis* subsp. *lactis*. Class I is being further subdivided into Ia and Ib. Class Ia bacteriocins, which include nisin, consist of cationic and hydrophobic peptides that form pores in the target membranes and have a flexible structure compared to class Ib. Class Ib bacteriocins, which are globular in nature, have no net negative charge (Altena *et al.*, 2000) and they exert their action by interfering with essential enzymatic reactions of sensitive bacteria (Deegan *et al.*, 2006).

Class II (small heat stable peptides):

Most of the new bacteriocins belong to the class II bacteriocins which are small (30–100 amino acids, < 10 kDa), heat-stable and commonly unmodified. It has been shown that several LAB produce multiple bacteriocins (2–3 bacteriocins) while most bacteriocin producers synthesize only one bacteriocin. The production of some class II bacteriocins (plantaricins of *Lactobacillus plantarum* C11 and sakacin P of *Lactobacillus sake*) have been shown to be transcriptionally regulated through a signal transduction system which consists of three components: an induction factor (IF), histidine protein kinase (HK) and a response regulator (RR) (Nath *et al.*, 2014).

They are also further subdivided into IIa and IIb, Class IIa includes pediocin PA-1 (Venema *et al.*, 1997) and sakacin P having anti-listerial activity with a conserved N-terminal sequence Tyr-Gly-Asn-Gly-Val and two cysteines forming S-S bridge in the N-terminal half of the peptide.

Class IIb:

Bacteriocins composed of two different and no sequence similarities between complementary peptides and are formed by a complex of two distinct peptides. These peptides have little or no activity. Examples are lactococcin G and plantaricins EF and JK. In this it needs both peptides to be fully active. The primary amino acid sequences of the peptides are different. Though each one is encoded by its own adjacent genes, only one immunity gene is needed (Cleveland *et al.*, 2001).

Class IIc:

Small peptides, heat-stable, which are transported by leader-peptides. It includes divergic in A and acidocin B.

Class III (Large heat labile bacteriocins):

These are big peptides, heat labile proteins with molecular weight over 30 kDa. It includes helveticins J (Joerger and Klaenhammer, 1986) and helveticins 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 nonlantibiotics (Cintas *et al.*, 2001).

Class IV (Circular peptides):

They include bacteriocins that form large complexes with other chemical moieties, carbohydrates or lipids required for activity. Presently, no such bacteriocins have been purified and it is believed that the reason is formation of complexes with other macromolecules in the crude extract due to their cationic and hydrophobic properties. The majority of bacteriocins produced by bacteria associated with food belong to classes I and II.

Mode of action of bacteriocin :

Different mechanisms of action have been proposed for bacteriocins such as: alteration of enzymatic activity, inhibition of spore germination and inactivation of anionic carriers through the formation of selective and non-selective pores (Abee, 1995; Martinez and De Martinis, 2006).

Bacteriocins, particularly lantibiotics, inhibit target cells by forming pores in the membrane, depleting the transmembrane potential ($\Delta\psi$) and /or the pH gradient, resulting in the leakage of cellular materials. In order to form pores by nisin, target cells require ($\Delta\psi$) (inside negative) and ΔpH (inside alkaline) has been suggested by the early studies. Bacteriocins are positively charged molecules with hydrophobic patches may be inefficient to inhibit Gram negative organisms because the outer membrane hinders the site for bacteriocin action, which is the cell membrane (De Martinis *et al.*, 2001 and Morisset *et al.*, 2004). Electrostatic interactions with negatively charged phosphate groups on target cell membranes are thought to contribute to the initial binding with the target membrane. It is likely that the hydrophobic

portion inserts into the membrane, forming pores. 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 and the “wedge” model, after a critical number of nisin molecules associate with the membrane, they insert concurrently, forming a wedge (Cleveland *et al.*, 2001).

LAB bacteriocins can work via different mechanisms to exert an antimicrobial effect, but the cell envelope is generally the target. The initial electrostatic attraction between the target cell membrane and the bacteriocin peptide is thought to be the driving force for subsequent events (Deegan *et al.*, 2006). Bacteriocins may possess a bactericidal or bacteriostatic mode of action on sensitive cells, this distinction being greatly influenced by several factors such as bacteriocin dose and degree of purification, physiological state of the indicator cells and experimental conditions (Cintas *et al.*, 2001). According to Jack *et al.* (1995), at least for the non-lanthionine-containing bacteriocins, the increased antibacterial activity observed at low pH may be the result of any one of a number of factors, including the following: (i) aggregation of hydrophilic peptides is less likely to occur, and, thus, more molecules should be available to interact with sensitive cells; (ii) fewer molecules will remain bound to the wall, making more molecules available for bactericidal action; (iii) hydrophilic bacteriocins may have an enhanced capacity to pass through hydrophilic regions of the cell wall of the sensitive bacteria; and (iv) interaction of the non-lanthionine-containing bacteriocins with putative membrane receptors may be inhibited at higher pH values.

Several features of the mode of action of the non-lanthionine-containing bacteriocins of gram positive bacteria require further explanation: (i) the reason why, for two sensitive strains, one undergoes lysis following treatment with a particular bacteriocin while the other is not known; (ii) for a bacteriocin to come into contact with the cytoplasmic membrane of sensitive cells, the molecules must firstly pass through the cell wall; the mechanism of this translocation remains to be understood; and, finally, (iii) there is evidence that non-lanthionine containing bacteriocin molecules may be adsorbed on the surface of most gram-positive bacterial cells, including sensitive, resistant, and producer strains;

the influence of this is not yet fully understood (Jack *et al.*, 1995).

Stability :

Some studies of characterization of bacteriocins show that these molecules can be active under certain ranges of temperature and pH. Sensibility to proteolytic enzymes evidences the proteinaceous characteristic of bacteriocins (De Martinis *et al.*, 2003). Torodov and Dicks, 2004, reported that complete inactivation or significant reduction in antimicrobial activity of the bacteriocins ST28MS and ST26MS produced by *Lactobacillus plantarum* isolated from molasses was observed after treatment with proteinase K, pronase, pepsin and trypsin. These bacteriocins remained stable after incubation for 2h at pH values between 2.0 and 12.0. No decrease in antibacterial activity was recorded after 90 min at 100°C or 20 min at 121° C. The thermotolerance feature might be related to the molecular structure of the bacteriocin, usually composed by small peptides without tertiary structure.

Requirements and regulatory status for bacteriocins:

In general, the following features should be considered when selecting bacteriocin-producing strains for food applications (Ananou *et al.*, 2007):

- The producing strain should preferably have GRAS status.
- Depending on the application, the bacteriocin should have a broad spectrum of inhibition that includes pathogens or else high specific activity.
- Thermostability.
- Beneficial effects and improved safety.
- No adverse effect on quality and flavour.

It is critical in some countries to distinguish bacteriocins from antibiotics since regulations often prohibit antibiotics in food (Wessels *et al.*, 1998). The use of bacteriocin-producing starter cultures as ingredients may not require special consideration in many countries (e.g. USA) if the micro-organism is GRAS. However, if a purified bacteriocin is used as a food preservative, the substance must be approved as GRAS, and for approval to be granted, the bacteriocin must be genetically and chemically identified and characterised, and its use and efficacy must be shown; the manufacturing process must be described and assays used for quantification and standardization of the peptide must

be shown as well. Toxicological data and the fate of the molecule after ingestion are also required.

Applying bacteriocins in food preservation :

The strategies for the application of LAB and/or bacteriocins in food are diverse:

- Inoculation of food with LAB (starter cultures or protective cultures) where bacteriocins are produced *in situ*.
- Use of food previously fermented with the bacteriocin-producing strains as an ingredient in the food processing (Nisaplin™, Microgard™, Alta™2341).
- Addition of purified or semipurified bacteriocins. The purified bacteriocins are considered additives and always require express authorization for their use (Fields, 1996).

To the date, the only commercially produced bacteriocins are the group of nisins produced by *Lactococcus lactis*, and pediocin PA-1, produced by *Pediococcus acidilactici* (Schobitz *et al.*, 2006). Minimally processed refrigerated foods have been gaining consumer acceptance in the last years due to their natural appeal. However, the microbiological safety of these foods is of concern due to the possible presence of non-proteolytic toxic strains of *Clostridium botulinum*, able to grow at 4°C, and the post-processing contamination with psychrophilic pathogens.

Food applications of bacteriocins :

Application of bacteriocins in dairy products :

Bacteriocins have wide applications in dairy industry especially during the fermentation of the products. Many researchers have demonstrated the effectiveness of nisin and/or nisin-producing strains against pathogenic bacteria such as *Clostridium botulinum* in cheese (Hirsch *et al.*, 1951) and against *L. monocytogenes* in cheeses such as Camembert (Sulzer, and Busse, 1991 and Maisnier-Patin *et al.*, 1992), Ricotta (Davies *et al.*, 1997), and Manchego (Nunez *et al.*, 1997). The lytic ability of bacteriocins like nisin and lactacin 3147 might be explored in the acceleration of cheddar cheese ripening. Cell lysis of the starter culture is advantageous for improved flavour development (Guinane *et al.*, 2005). The level of nisin used depends on food composition, spore load, required shelf-life and temperature during storage (Hirsch *et al.*, 1951). Other bacteriocins have been tested in milk and dairy products,

such as pediocin AcH in milk and Cheddar and Munster cheeses against *L. monocytogenes*, *S. aureus*, and *E. coli* O157:H7 (Buyong *et al.*, 1998 and Rodríguez *et al.*, 1995), lacticin 3147 against undesirable LAB, *L. monocytogenes* and *B. cereus* in Cheddar, Cottage cheese and yogurt (Ross *et al.*, 2002; Ryan *et al.*, 1996; McAuliffe *et al.*, 1999; Morgan *et al.*, 2001) and enterocin AS-48 against *B. cereus*, *S. aureus* and *L. monocytogenes* in milk and Manchego cheese (Muñoz *et al.*, 2004; Muñoz *et al.*, 2007). Zottola *et al.* (1994) used nisin-containing cheddar cheese that had been made with nisin-producing lactococci as an ingredient in pasteurized process cheese or cold pack cheese spreads. The shelf-life of the nisin-containing pasteurized process cheese (301 and 387 IU nisin/g) was significantly greater than that of the control cheese spreads.

Applications in meat products :

When evaluating a bacteriocin-producing culture for sausage fermentation and/or biopreservation, one must bear in mind that meat and meat products are complex systems with a number of factors influencing microbial growth and metabolite production. Therefore, the influence of formula and fermentation technology on the performance of bacteriocin-producing cultures needs to be assayed. The most-studied bacteriocins in meat and meat products include nisin, enterocin AS-48, enterocins A and B, sakacin, leucocin A, and especially pediocin PA-I/AcH, alone or in combination with several physico-chemical treatments, modified atmosphere packaging, high hydrostatic pressure, (HHP), heat, and chemical preservatives, as an additional hurdle to control the proliferation of *L. monocytogenes* and other pathogens (Cleveland *et al.*, 2001; Nielsen *et al.*, 1990; Cutter and Siragusa, 1994; Aymerich *et al.*, 2000; Garriga *et al.*, 2002; Ananou *et al.*, 2005a; Ananou *et al.*, 2005b). Furthermore, several bacteriocinogenic LAB have been used as bioprotective cultures for food manufacturing processes in attempts to control these pathogens (Cleveland *et al.*, 2001; Vandenberg, 1993 and Campanini, 1993). The data available on the use of nisin in cured and fermented meat are equivocal. Compared to dairy products, nisin use in meat products has not been very successful because of its low solubility, irregular distribution, and lack of stability. Pediocin PA-I/AcH is more suitable for use in meat and meat products than nisin; however, *P. acidilactici* is not an indigenous meat strain

(Cleveland *et al.*, 2001).

Applications in vegetable products :

Tests of bacteriocins in vegetable products include nisin in tinned vegetables and fruit juices (Delves-Broughton, 1999; Alpas and Bozoglu, 2000 and Komitopoulou *et al.*, 1999), pediocin PA-I/AcH in salad and fruit juice (Cleveland *et al.*, 2001 ; Alpas and Bozoglu, 2000 and Vedamuthu *et al.*, 1992), and enterocin AS-48 against *B. cereus* in rice and vegetables (Cobo Molinos *et al.*, 2005 and Grande *et al.*, 2006) and in fruit juices against other pathogens such as *E. coli* O157:H7, *S. aureus* and the spoilage bacterium *Alicyclobacillus acidoterrestris* (Ananou *et al.*, 2005c; Grande *et al.*, 2006 and Grande *et al.*, 2005).

Applications in fish :

The deterioration of fresh fish is generally caused by Gram-negative microorganisms; however, in vacuum-packed fresh fish and seafood, pathogenic organisms such as *Clostridium botulinum* and *L. monocytogenes* can also cause problems. The combination of nisin and Microgard reduced the total aerobic bacteria populations of fresh chilled salmon, increased its shelf-life, and also reduced the growth of inoculated *L. monocytogenes* in frozen thawed salmon (Zuckerman and Ben Avraham, 2002). Nykänen *et al.*, 1989 demonstrated the synergistic effect of combination lactic acid, sodium chloride, and/or nisin in rainbow trout, and more recently (Vázquez *et al.*, 2005) showed the effect of LAB cultures on pathogenic microorganism control in fish. Listerial control was achieved in cold-smoked salmon using *Carnobacterium divergens* or *L. sakei*.

Canned food products :

Alcoholic beverages :

The insensitivity of yeasts to nisin allows its use to control spoilage LAB in beer or wine. It can maintain its activity during fermentation without any effect on growth and fermentative performance of brewing yeast strains and with no deleterious effect on taste. It can therefore be used to reduce pasteurization time- temperature combination and can increase the shelf-life of beers (Bali *et al.*, 2012). Nisin can also be used to reduce the amount of sulphur dioxide used in wine making to control bacterial spoilage (Todorov *et al.*, 2003).

Future trends :

Bacteriocins represent one of the best-studied microbial defence systems. Foods preserved with biopreservative is getting popular due to greater consumer awareness and concern regarding synthetic chemical additives, this has leadscientists forthe isolation of new bacteriocins from food products and the various fermented products like milk products, vegetables, fruits, cereals, meat are few of the examples. There is a need to explore more micro-organisms producing novel bacteriocins with unique preservation characters, bacteriocin modifications with protein engineering, construction of food grade vectors, regulation and expression of heterologous proteins, modification and control of organoleptic properties of food items is also the thrust area of research. The isolation of novel bacteriocins from different strains and their combination with some suitable physical preservation techniques like low temperature, hurdle technology and mild thermal treatment, etc. can be proven by promising to provide safer and healthier food products by food processing industry.

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