Bacteriocins: A natural way to combat with pathogens

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Preservation of foods by natural and microbiological methods may be a satisfactory approach solving economic losses due to microbial spoilage of raw materials and food products, to reduce the incidence of food borne illnesses, and to meet the food requirements of the growing world population. Since many people are concerned about the safety of chemical preservatives are questioned with regard to their safety, the potential applications of bacteriocins from lactic acid bacteria (LAB) in food and health care have received increasing attention in recent years. Proteinaceous nature of bacteriocins implies a putative degradation in the gastro-intestinal tract of man, suggesting that some bacteriocins could be used as food preservatives such that bacteriocins have a wide antibacterial spectrum with potential applications in foods. Today nisin and pediocin have been used to inhibit some pathogens successfully and there are lots of research focusing on the finding new bacteriocins or new methods to use them in food systems.

Keywords bacteriocin; lactic acid bacteria; pathogen inhibition

1. The importance of bacteriocins among the natural antimicrobial compounds

Consumers may be more concerned about safety in food than in any other products, including medicines. Undoubtedly the major threat to food safety is the emergence of pathogens such as *Listeria monocytogenes*, *Escherichia coli* O157:H7, *Campylobacter jejuni*, *Yersinia enterocolitica* and *Vibrio parahemolyticus* as foodborne microorganisms has been related to the increase of outbreaks (Castellano et al. 2008). Animals and plants have developed a wide variety of defensive strategies against bacteria which can cause illness and death. Apart from physical barriers, several antimicrobial substances are produced by the innate immune system to fight bacterial infections (Ruiz-Rodriguez et al. 2013). The use of antibiotics in intensive animal production poses the additional risk of bacterial resistance, which constitutes a microbiological hazard rather than a strictly chemical residue one (Castellano et al. 2008). Further, pathogens resistant to most conventional antibiotics thus there is a need to discover novel antimicrobials and anti-infective agents and develop innovative strategies to combat them (Naghmouchi et al. 2013). The preservation of foods by natural and microbiological methods may be a satisfactory approach to reduce the incidence of food borne illnesses, solving economic losses due to microbial spoilage of raw materials and food products, and to meet the food requirements of the growing world population (Galvez et al. 2008, Altuntas et al. 2010).

Biopreservation refers to the extended storage life and enhanced safety of foods using their natural or controlled microflora and/or their antibacterial products (Galvez et al. 2008). Gene-encoded, ribosomally synthesized antimicrobial peptides are widely distributed in nature, being produced by bacteria, plants, and a wide variety of animals, including humans. The peptides are often cationic and amphiphilic or hydrophobic, and many of them kill bacteria by permeabilizng the target cell membrane. The peptides may be developed into new and useful antimicrobial additives and drugs. An example of this is the antimicrobial peptides bacteriocins, which are produced by lactic acid bacteria (Uteng et al. 2002). Since many people are concerned about the safety of chemical preservatives are questioned with regard to their safety, the potential applications of bacteriocins from lactic acid bacteria in food and health care have received increasing attention in recent years (Papagianni and Anastasiadou 2009). Bacteriocins have a wide antibacterial spectrum with potential applications in foods, such as meat and fish products, fruits and vegetables, cereals and beverages (Ivanova et al. 2000, Cleveland et al. 2001).

Although many bacteria can produce bacteriocins, those produced by LAB are of particular interest to the food industry, since these bacteria have GRAS (generally regarded as safe) status (Barefoot and Nettles 1993, Elegado et al. 1997, Anastasiadou et al. 2008, Altuntas et al. 2010). LAB have been used in food production as an effective method for extending shelf life of food stuffs by simple fermentation (Galvez et al. 2008). *Lactococcus*, *Streptococcus*, *Pediococcus*, *Leuconostoc*, *Lactobacillus* and *Carnobacterium* are the genera most commonly used as starter cultures in the fermentation processes of milk, meat and vegetable products (Hastings and Stiles 1991, Martinez-Cuesta et al. 2001, Albano et al. 2007). These bacteriocin-producing bacteria are probably among the most promising natural food biopreservatives (Atanassova et al. 2001, Leroy et al. 2003). Use of bacteriocins, or the organisms which produce them, or both, could be attractive to the food industry because of addressing to the consumer demand for natural products (Montville and Winkowski 1997).

Over the last 2 decades, a variety of bacteriocins, produced by bacteria that kill or inhibit the growth of other bacteria, have been identified and characterized biochemically and genetically (Chen and Hoover 2003). Those produced by LAB have been of particular interest because of their existing and potential ability to act as biopreservatives (Duffes et al. 2000, Anastasiadou et al. 2008, Altuntas et al. 2012).

Bacteriocins of LAB are usually antagonistic to genetically closely related organisms and Gram-positive bacteria but in the last decade, some research papers have demonstrated that certain bacteriocins are also active against certain Gram-negative bacteria, such as *Escherichia coli* and *Salmonella* Typhimurium and interestingly against *Campylobacter jejuni*, the major cause of gastroenteritis worldwide. Furthermore, bacteriocins can block the reproduction of some viruses. (Murua et al. 2013)

Bacteriocins have been known for approximately 90 years and among them nisin widely studied bacteriocin was discovered in 1928 and it is the first bacteriocin used in food systems as biopreservative (Gürakan 2007).

More than 300 different bacteriocins have been described for the genera *Lactobacillus*, *Lactococcus*, *Leuconostoc*, *Pediococcus* and *Enterococcus*. They are generally low molecular- weight proteins that gain entry into target cells by binding to cell surface receptors and which bactericidal mechanisms vary, including pore formation, degradation of cellular DNA, disruption through specific cleavage of 16S rRNA and inhibition of peptidoglycan synthesis (Murua et al. 2013).

Proteinaceous nature of bacteriocins implies a putative degradation in the gastro-intestinal tract of man, suggesting that some bacteriocins could be used as food preservatives (Vaucher et al. 2011). Due to their resistance to temperature and low pH, the bacteriocins are digested by human and animal peptidases, thus avoiding resistance and problems associated to the presence of residues in feed and food (Javed 2009).

Several classes of bacteriocins have been described based on their size, posttranslational modifications, production and heat liability. Klaenhammer classified the LAB bacteriocins into four major classes (Klaenhammer 1988). Class I includes the lantibiotics, bacteriocins that possess the characteristic lanthionine moiety and are often produced by lactic acid bacteria. Class II excludes the lantibiotics and contains heat stable bacteriocins. This class is also subdivided into sections a, b and c. Class III contains larger (430 kDa) heat-liable bacteriocins and Class IV contains bacteriocins that are modified with either lipid or carbohydrate components (Gray et al. 2006). It has recently been proposed that circular LAB bacteriocins should be regarded as class V bacteriocins which are included in the nonlanthionine-containing class II category. Nisin, which so far is the only bacteriocin widely used in the food industry, and lacticin 481 are representatives of subgroups of Class I. Class II bacteriocins which are nonlantibiotic heat-stable peptides and are of particular interest because of their antilisterial activity contains pediocin PA1/AcH and the closely related compound coagulin (Hindre et al. 2003).

Bacteriocins are mostly synthesized as biologically inactive prepeptide molecule which carries an N-terminal leader peptide which is attached to the C-terminal propeptide. Prepeptide formation, reaction modification, proteolytic cleavage of the leader peptide, and the translocation of the modified prepeptide or mature propeptide across the cytoplasmic membrane are the most important biosynthetic pathways for lantibiotics. Prior to or during or after export from the cell, the cleavage of the leader peptide may happen (Kanmani et al. 2013).

The mode of action of bacteriocins is not yet fully understood but the model membrane studies with nisin have shown that lipid II acts as a docking station. After binding, nisin wedges itself into the cell membrane to form short-lived pores which disturb the integrity of the cytoplasmic membrane and causes the efflux of ions and other cell components. At high concentrations of nisin, pore formation may ocur in the absence of lipid II, provided the cell membrane contains at least 50% negatively charged phospholipids. Under these conditions, the positively charged C-terminus of nisin is important for initial binding and antimicrobial activity. Mersacidine and the antibiotic vancomycin also bind to lipid II, but to a different part of the molecule (Bauer et al. 2005). In summary, bacteriocins kill sensitive bacteria by forming pores on the cytoplasmic membrane or by inhibiting synthesis of the cell wall (Diep et al. 2006). Whereas, normally the cells producing the bacteriocins are immune to its antagonistic action and therefore might enjoy a competitive advantage over sensitive bacteria inhibiting the same ecological niche (Aslam et al. 2011).

Bauer et al. (2005) reported that genes encoding bacteriocin production are often located on plasmids. It was also suggested by Tagg et al. (1976), that genes coding for bacteriocin produced by various LABs are plasmid linked and the Bac+ phenotype can be eliminated by using standard technique for plasmid curing.

The traditional determination of the antagonism of a bacteriocin-producing strain against a sensitive strain, generally indicated as “producer” and “indicator”, respectively, can be performed in different ways. In general, the methods most frequently used are the agar-spot deferred test and the well diffusion assay (Schillinger and Lücke 1989). Bacteriocin activity of the supernatants is commonly evaluated by the critical dilution assay of defined as the reciprocal of the highest dilution showing clear inhibition of the indicator strains and is expressed as activity units per millilitre (AU/ml). Agar based antagonistic assays may be replaced by quicker tools for bacteriocin detection such as by means of the matrix assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS). Furthermore, PCR methods have also been used to detect genes coding for bacteriocins in pure cultures and fermentation broth (Settanni and Corsetti 2008).

Among the bacteriocins, nisin, the bacteriocin commercially available as a bio-preservative, is produced by numerous *Lactococcus lactis* strains and has been approved in over 50 countries for many applications in a large range of food products, particularly in cheese manufacturing. Two naturally occurring nisin variants namely nisin A and nisin Z that differ in a single amino acid residue but showing similar activities have been described (Javed 2009).

Nisin comprises 34 amino acids, including the post-translationally modified amino acids thioether-bridged lanthionine and 3-methyllanthionine and unsaturated 2,3-didehydroalanine and 2,3-didehydrobutyrine. Nisin exhibits antibacterial activity against a wide range of Gram-positive bacteria, including LAB and bacteria of the genera *Listeria*, *Staphylococcus* spp., *Bacillus* spp., and *Clostridium* spp (Fujita et al. 2007). Nisin has also been considered for use for treatment of gastric Helicobacter infections and/or ulcers (Uteng et al. 2002). The successful development of nisin from an initial biological observation through regulatory approval for commercial applications, is a model that has stimulated new contributions in the field of bacteriocin research (Parada et al. 2007). However, its low stability at neutral and alkaline pH values limits the range of its use, and this is also a drawback of several other LAB bacteriocins (Fujita et al. 2007).

Outside of nisin, it is well known that several strains of *Pediococcus acidilactici* and *P. pentosaceus*, significant members of LAB, were found to produce pediocins (Biswas et al. 1991, Elegado et al. 1997, Gurira and Buys 2005, Papagianni and Anastasiadou 2009). Pediocin-like bacteriocins (Class II LAB bacteriocins) are known as small (<10 kDa), heat stable, non-lanthionine containing, membrane active peptides (Deraz et al. 2005, Xiraphi et al. 2008). Pediocin AcH or PA-1 was first and most thoroughly characterized bacteriocin among Class IIa bacteriocins, and it is produced by several *P. acidilactici* strains. One of the most important characteristics of pediocin-like bacteriocins is their high antimicrobial activity against *Listeria monocytogenes* (Gravesen et al. 2002, Schneider et al. 2006, Cosansu et al. 2007, Albano et al. 2007, Altuntas et al. 2010). *Listeria monocytogenes* is a Gram-positive bacterium that is capable of growth at low temperatures and is known for its ability to adapt to various environmental conditions, such as acidity and high salt. Because of this, as well as its wide distribution in the environment and food, it is of great concern to the food industry. *L. monocytogenes* can cause sporadic or epidemic cases of food-borne listeriosis, which can lead to high fatality rates in the elderly, pregnant women, newborns, and immunocompromised individuals (Liu et al. 2013).

More than 20 members of the pediocin-like bacteriocins have been identified to date. These peptides can be structurally divided into a highly conserved hydrophilic N-terminal domain and a relatively variable hydrophobic C-terminal domain (Tominaga and Hatakeyama 2006). A major concern for the food industry is the psychrotrophic pathogenic microorganism, ubiquitous in nature, like *Listeria monocytogenes*. Its ability to survive in various environmental conditions, such as refrigeration, at pHs as low as 3.6 in foods, in salt concentrations up to 10%, significantly contributes to its hazard status. The capability of lactic acid bacteria to produce bacteriocins with anti-listerial activity has gained much interest in improving the safety of various foods (Xiraphi et al. 2008, Sofos and Geornaras 2010, van Kuijk et al. 2011). Isolation and purification of new bacteriocins will always prove beneficial. Characterization of the purified form of bacteriocins will provide information on economically viable mass production along with generating manipulations to improve bacteriocin properties and their effectiveness (Elegado et al. 1997). In depth the characterization of bacteriocins is dependent upon their purification. Elucidation of their biochemical structure requires homogeneity as well as an adequate yield of protein. There are lots of studies focused on purification methods of bacteriocins; however, it is still a necessity to develop new alternative methods. This requirement may be due to the extremely heterogeneous nature of bacteriocins (Carolissen-Mackay et al. 1997).

The past few years have seen the emergence of class IIa bacteriocins as one of the most interesting groups of antimicrobial peptides for use in food preservation and medicine, as antibiotic complements in treating infectious diseases or antiviral agents (Drider et al. 2006) or therapeutic agents (Jasniewski et al. 2008). The bacteriocins produced by *Pediococcus* spp. are classified as class IIa bacteriocins and have high antimicrobial activity, especially against *L. monocytogenes* (Cintas et al. 1998, Kim et al. 2000, Mattila et al. 2003, Rodriguez et al. 2005, Cosansu et al. 2007). Another important characteristic of these bacteriocins is their narrow inhibitory spectrum which prevents inhibition of starter cultures more than class I bacteriocins such as nisin (De Carvalho et al. 2006, Schneider et al. 2006).

2. Bacteriocins produced by lactic acid bacteria

LAB are characterized as Gram-positive cocci or rods, non-aerobic but aerotolerant, able to ferment carbohydrates for energy and lactic acid production (Parada et al. 2007). In recent years, increasing interest has been shown in biopreservation due to great consumer demands for the reduction of chemical preservatives in foods (Gürakan 2007). LAB have been essential for millennia in the case of fermented foods due to their wide presence in nature and their tolerance to acid and oxygen environments. The bacteria belonging to LAB have been used in the production of fermented foods for ages since they can provide desirable taste, flavor and texture (Guzel-Zeydim and Ekinci 2007, Settanni and Corsetti 2008, Fadda et al. 2010, Chen et al. 2013, Sawa et al. 2013). The genera *Lactobacillus*, *Leuconostoc*, *Pediococcus* and *Streptococcus* are well known and widely studied group of lactic acid bacteria. LAB produce some antimicrobial compounds including; organic acids, hydrogen peroxide, carbon dioxide, diacetyl, acetaldehyde, reuterin, bacteriocins (Gürakan 2007).

These microorganisms are found in milk, meat and fermented products, as well as in fermented vegetables and beverages inhibiting the growth of pathogenic and deteriorating microorganisms, maintaining the nutritive quality and improving the shelf life of foods. 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. *Lactobacillus acidophilus*, *L. plantarum*, *L. casei*, *L. casei rhamnosus*, *L. delbrueckii bulgaricus*, *L. fermentum*, *L. reuteri*, *Lactococcus lactis lactis*, *Lactococcus lactis cremoris*, *Bifidobacterium bifidum*, *B. infantis*, *B. adolecentis*, *B. longum*, *B. breve*, *Enterococcus faecalis*, *Enterococcus faecium*, are some of the most common species and some strains are recognized as probiotics (Parada et al. 2007).

Bacteriocins produced by some lactic acid bacterial strains have been shown to exhibit activity against several spoilage bacteria and food-borne pathogens, including *Staphylococcus aureus*, *Enterococcus faecalis*, *Clostridium botulinum*, and *Listeria monocytogenes* (Leroy and De Vuyst 1999). Numerous studies have been conducted on the environmental conditions and medium composition requirements for optimized bacteriocin production by lactic acid bacteria. It is generally observed that lactic acid bacteria produce bacteriocin in a growth-associated way. However, good cell growth does not necessarily result in sufficient bacteriocin production (Leroy and De Vuyst 2005).

The increasing interest in such antimicrobial peptides as natural food additive candidates, has stimulated the isolation and characterization of many novel peptides from bacteria that have traditionally been used by humans in food applications. LAB and their metabolites have been consumed in cultured foods with no adverse effects in high quantities by countless generations of people (Deraz et al. 2005). Today antibiotics are restricted for use in foods and feeds, and bacteriocins produced by LAB are an interesting group of biomolecules with antimicrobial properties that may represent a good alternative. (Parada et al. 2007). From another perspective, bacteriocin production is often proposed as a beneficial characteristic of probiotics that may contribute to host protection against gastrointestinal pathogens. Moreover, bacteriocin production may facilitate the establishment of a strain in the competitive environment of the gut. (Lavilla-Lerma et al. 2013)

However many bacteriocins and producer strains are known, nisin (known as Nisaplin^{TM}) and pediocin PA-1 (known as ALTA^{TM}2431) produced by LAB are the only bacteriocins allowed to use for biopreservation of foods (Gürakan 2007).

3. Applications of bacteriocins in food systems

Before a bacteriocin is considered for application in food, information on its antibacterial spectrum, biochemical and genetic characteristics, effectiveness in food systems, and regulatory implications should be known. Most wanted one is their safety studies; they should not possess any virulence or antibiotics resistance. Another important factor to consider will be the economical aspects or cost of using a bacteriocin in foods (Javed 2009).

On the assumption that a specific bacteriocin will have its own unique properties and usefulness in targeting microbial pathogens, isolation and purification of new bacteriocins will always prove beneficial. In-depth characterization of the purified form of new isolates will provide infomation for economically viable mass production and downstream processing together with useful data for making manipulations to improve bacteriocin properties and effectiveness and to hasten consumer acceptability of bacteriocin-treated products (Elegado et al. 1997). Isolation, concentration, and purification of most bacteriocins are done by salt precipitation followed by various combinations of gel filtration, ion-exchange chromatography, hydrophobic-interaction chromatography, and reverse-phase high-performance liquid chromatography from culture supernatants. Many protocols for purification of bacteriocins have many disadvantages such as being complex and time consuming which usually resulted in low protein yields. To overcome this problem, reliable methods such as cation-exchange chromatography based on the utilization of hydrophobic C-terminal domains of Class IIa bacteriocins have been developed (Guzel-Zeydim and Ekinci 2007). One of the main drawbacks for application of bacteriocins in foods is the difficulty in obtaining large-scale preparations suitable for assays and application at pilot or industrial scale. A common approach has been to obtain partially purified concentrates (usually in the form of a lyophilized powder) after growth of the bacteriocin-producing strain in a milk-based substrate.

Currently, non-thermal preservation methods are of growing interest as alternative treatments, especially high intensity pulsed electric fields (HIPEF), high pressure (HP) and the addition of natural antimicrobial substances such as lactic acid and other end products of LAB metabolism, including hydrogen peroxide, diacetyl, acetoin and other organic acids. Bacteriocins are thougt as one promising method among antimicrobial agents (Altuntas et al. 2012)

Bacteriocin applications are widely studied in cheese production systems. In one of these studies, the effect of high-pressure (HP) treatments combined with bacteriocins of LAB produced in situ on the survival of *Escherichia coli* O157:H7 in cheese was investigated. Seven different bacteriocin-producing LAB were added at approximately 10^6 CFU/ml as adjuncts to the starter and cheeses were pressurized. Pressurization at 300 MPa on day 2 and addition of lacticin 481, nisin A, bacteriocin TAB 57, or enterocin AS-48-producing LAB were synergistic and reduced *E. coli* O157:H7 counts to levels below 2 log units in 60-day-old cheeses. Pressurization at 300 MPa on day 50 and addition of nisin A, bacteriocin TAB 57, enterocin I, or enterocin AS-48-producing LAB completely inactivated *E. coli* O157:H7 in 60-day-old cheeses. The application of reduced pressures combined with bacteriocin-producing LAB is a feasible procedure to improve cheese safety (Rodriguez et al. 2005).

Among the natural isolates of LAB from homemade cheeses, bacteriocin producers were found in both lactococci and lactobacilli. *Lactococcus lactis* subsp. *lactis* BGMN1–5 was found to produce three narrow spectrum class II heat-stable bacteriocins. In addition to bacteriocin production, BGMN1–5 synthesized a cell envelope-associated proteinase (CEP) and shows an aggregation phenotype. Another isolate, *L. lactis* subsp. *lactis* BGSM1–19 produces low molecular mass (7 kDa) bacteriocin SM19 that showed antimicrobial activity against *Staphylococcus aureus*, *Micrococcus flavus* and partially against *Salmonella paratyphi* (Topisirovic et al. 2006).

Yogurt starter cultures commonly contains *Streptococcus thermophilus* which have been well studied for the production of the bacteriocins, is a heterogeneous group of peptide which have wide spectrum of activity against bacteria. *S. thermophilus* bacteriocins have been reported to have a broad inhibitory spectrum against several bacteria *Listeria monocytogenes*, *Salmonella* Typhimurium, *Escherichia coli*, *Yersinia pseudotuberculosis* and *Yersinia enterocolitica*, *Clostridium tyrobutyricum* (Aslam et al. 2011).

In the wake of recent outbreaks associated with *Listeria monocytogenes* in ready-to-eat foods and an increasing desire for minimally processed foods, there has been a burgeoning interest in the use of natural antimicrobials by the food industry to control this pathogen. The two most effective antimicrobial formulations for smoked salmon, 0.25% sodium diacetate and 2.4% sodium lactate / 0.125% sodium diacetate, were able to inhibit the growth of *L. monocytogenes* during the 3 weeks of storage. Surface application of 2.4% sodium lactate / 0.125% SD was the most effective treatment for smoked salmon fillets which inhibited the growth of *L. monocytogenes* for 4 weeks. These antimicrobial treatments could be used by the smoked salmon industry in the U.S. and Europe in their efforts to control *L. monocytogenes* as they are effective against even the most antimicrobial-resistant strains tested in this study (Neetoo et al. 2008).

The increasing popularity of salad bars offering freshly cut lettuces, which are also found in supermarkets and convenience stores, has introduced new environments which support the growth of food-borne pathogens including *Listeria monocytogenes*. The effect of washing with bacteriocin-containing solutions on survival and proliferation of *Listeria monocytogenes* was evaluated in fresh-cut lettuce packaged in macro-perforated polypropylene bags and stored for 7 days at 4°C. Washing fresh-cut lettuce with these solutions decreased the viability of *Listeria monocytogenes* by 1.2–1.6 log units immediately after treatment, but, during storage at 4°C, bacteriocin treatments only exerted minimal control over the growth of the pathogen. Natural microbiota were little affected by bacteriocins during storage (Allende et al. 2007).

A complementary approach, combining X-ray photoelectron spectroscopy (XPS) and time-of-flight secondary ion mass spectrometry (ToF-SIMS) was used to investigate the antimicrobial peptide nisin adsorption on hydrophilic and hydrophobic surfaces. The native low density polyethylene was used as hydrophobic support and it was grafted with acrylic acid to render it hydrophilic. XPS permitted to confirm nisin adsorption and to determine its amount on the surfaces. ToF-SIMS permitted to identify the adsorbed bacteriocin type and to observe its distribution and orientation behavior on both types of surfaces. Nisin was more oriented by its hydrophobic side to the hydrophobic substrate and by its hydrophilic side to the outer layers of the adsorbed peptide, in contrast to what was observed on the hydrophilic substrate. A correlation was found between XPS and ToF-SIMS results, the types of interactions on both surfaces and the observed antibacterial activity. Such interfacial studies are crucial for better understanding the peptides interactions and adsorption on surfaces and must be considered when setting up antimicrobial surface (Karam et al. 2013).

Pathogens are also threaten human health in biofilm forms in different areas such as the medical field, aquatic environment, food processing industries, etc. The formation of biofilms by some pathogenic bacteria such as *S.enteritidis*, *L. monocytogenes* and *Staph. aureus* have been reported. Biofilm is a functional consortium of microorganisms attached to the surface and is embedded in the extracellular polymeric substances (EPS) produced by the microorganisms. Biofilms due to special structure and EPS are more resistant to antimicrobial agents. Thus control of biofilm formation in food processing is important. Nisin is a peptidic bacteriocin that is used for biocontrol of biofilm formation. In a study the aim was to assess the effect of various concentration of nisin on biofilm formation of *Listeria monocytogenes*, *Staphylococcus aureus* and *Salmonella enteritidis*. The reduction percent of biofilms was obtained using microtiter plate method and ELISA reader machine. Also, bactericidal effect of nisin was determined by Triphenyl Tetrazolium Chloride. The results indicated that 4×10^3 IU/ml nisin is more effective on biofilm of *S. enteritidis* (87%) than *L. monocytogenes* (57%) and *Staph. aureus* (30%) with significant difference ($P<0.05$) (Mahdavi et al. 2007).

Differently from these studies, Selle and Klaenhammer (2013) demonstrated the specific role of bacteriocins in probiotic-mediated antagonism of pathogens in vivo in a mouse model challenged with *L. monocytogenes*. The study elegantly showed that salivaricin was solely responsible for the protective effect conferred by *L. salivarius* UCC118 in preventing infection and mortality. In light of these results, it remains essential to investigate the potential application of novel bacteriocins from *L. gasseri* in preventing infection from Gram-positive enteric pathogens in vivo. Bacteriocins isolated from *L. gasseri* may also be applied in food preservation, as their heat stability and extensive pH range may afford a suitable shelf life in products. Moreover, their broad-spectrum activity is conducive to antagonism of spoilage microorganisms and pathogens alike.

Predictive microbiology is a promising technique nowadays. Similarly Leroy and De Vuyst (2003) reported that predictive microbiology or a mathematical estimation of microbial behavior in food ecosystems may help to overcome this problem. In this study, a combined model was developed that was able to estimate, from a given initial situation of temperature, pH, and nutrient availability, the growth and self-inhibition dynamics of a bacteriocin-producing Lactobacillus sakei CTC 494 culture in (modified) MRS broth. Moreover, the drop in pH induced by lactic acid production and the bacteriocin activity toward *Listeria* as an indicator organism were modeled. Self-inhibition was due to the depletion of nutrients as well as to the production of lactic acid. Lactic acid production resulted in a pH drop, an accumulation of toxic undissociated lactic acid molecules, and a shift in the dissociation degree of the growth-inhibiting buffer components. The model was validated experimentally.

4. Bacteriocins in hurdle technology

New trends in food processing, product development and quality assurance are promoting intense research on alternative methods for food preservation. Most foods are thermally preserved by subjecting the products to boiling (or even higher) temperatures for a few seconds to several minutes. These high-energy treatments usually diminish cooking flavors, and cause loss of vitamins, essential nutrients, and food flavors in the product.To overcome or minimize such disadvantages, the concept of nonthermal treatments was born. Foods can be nonthermally processed by 1) irradiation, 2) high hydrostatic pressure (HHP), 3) the use of antimicrobials, bacteriocins, or chemicals, 4) ultrasound, 5) micro and ultrafiltration, and 6) electrical methods such as pulsed electric fields (PEF), light pulses (LP), and oscillating magnetic fields (OMF) (Barbosa-Canovas et al. 2005).

Hurdle technology refers to the concept of achieving control contamination of food by combining, a number of measures that would not individually be adequate for control. In this technique each individual control measure is considered as a hurdle (Guzel-Zeydim and Ekinci 2007).The hurdle system allows the production of high quality foods that are minimally processed, additive free, and microbiologically safe. Previous work has shown that many factors can influence the sensitivity of micro-organisms to pressure, such as magnitude of pressure, pressurization time and temperature, microbial types, cell growth phase, suspending media, and the presence of antimicrobialcompounds such as bacteriocins and lysozyme (Alpas and Bozoğlu 2000).

Physical treatments have also been shown to potentiate bacteriocin activity. For example, nonthermal treatment of high intensity pulsed electric field (HIPEF) can lead to microbial inactivation by the application of high voltage pulses, damaging the bacterial membrane and thus complementing the mode of action of bacteriocins. The observed synergy between bacteriocins and high hydrostatic pressure (HHP) has also been hypothesized to be a result of cumulative damage to the cytoplasmic membrane (Mills et al. 2011).

Bacteriocins are generally not active against Gram-negative bacteria because of their outer membrane, a barrier against certain hydrophobic solutes and macromolecules. The chelating agents such as EDTA can destabilize the lipopolysaccharide layer of outer membrane and make bacteriocins more effective on pathogen inhibition (Gürakan 2007). Treatment with metal-chelating agents as ethylenediaminetetraacetate (EDTA) generally results in removal by chelation of divalent cations from LPS layer. Since chelators are compounds able to sequester metal ions forming stable metal complexes, they chelate Ca^{2+} and Mg^{2+} ions from the outer membrane of Gram-negative cells, destabilizing its structure and altering its permeability making cells sensitive to hydrophobic peptides such as bacteriocins. (Belfiore et al. 2007). Furthermore, the application of hydrostatic pressure, heat, freezing or addition of ethyl maltol with bacteriocin have proven successful results (Guzel-Zeydim and Ekinci 2007). Castellano et al. (2011) was also reported LAB inactivation of Gram-negative pathogens on foods in combination with other hurdles or treatments would induce cell damage and partial disorganization of the outer membrane protective layer.

Organic acids can work well with bacteriocins as the increase in net charge of bacteriocins at low pH may facilitate bacteriocin translocation through the cell wall.In addition, the solubility of some bacteriocins may also be improved at low pH, facilitating diffusion. Combining two or more bacteriocins has also provided promising results, particularly if the bacteriocins belong to different grouping schemes targeting different cellular components (Mills et al. 2011).

High-pressure treatment (HPT), a nonthermal method of food preservation used for a wide variety of products (Garriga et al. 2002), could represent an alternative to milk pasteurization in the manufacture of raw milk cheeses, improving the microbiological quality while maintaining their sensory characteristics. Magnitude of the pressure, pressurization time and temperature, microbial species and strain, cell growth phase and suspending media affect the sensitivity of microorganisms to HPT. Bacteriocins or bacteriocin-producing lactic acid bacteria (BP-LAB) may reduce levels of *S. aureus* in contaminated foods. Nisin added to milk in white pickled cheese manufacture or produced in situ by a *Lactococcus lactis* subsp. *lactis* strain in a semi-hard cheese variety showed scarce bactericidal effect on *S. aureus*, but a complete elimination of the pathogen was achieved when nisin was added to process cheese spreads (Arques et al. 2005).

Many factors affect the sensitivity of microorganisms to HP treatments, including the magnitude of the pressure, the pressurization time and temperature, the microbial species and strain, the cell growth phase, and the suspending medium. (Rodriguez et al. 2005).

The behaviour of several foodborne bacteria inoculated in a meat model system with added bacteriocins (enterocins A and B, sakacin K, pediocin AcH or nisin) after pressurization (400MPa, 10min) and during chilled storage was investigated. Although *Staphylococcus* was the genus least sensitive to pressurization, the samples including nisin displayed lower and significantly different counts during the 4°C storage than the rest of the treatments. A greater inactivation of *Escherichia coli* in the presence of nisin was recorded, the number of survivors remained unchanged during storage at 4°C for 61 days (Garriga et al. 2002).

Foodborne bacterial spores are normally resistant to high hydrostatic pressure; however, at moderate pressure, they can be induced to germinate and outgrow. In a study performed by Kalchayanand et al. (2003), spores of the meat spoilage organism, Clostridium laramie alone or a mixture offour clostridial spores, *Clostridium sporogenes*, *Clostridium perfringens*, *Clostridium tertium*, and *Clostridium laramie*, were inoculated in roast beef in the presence of 5000 AU/g of bacteriocin-based biopreservatives. The roast beef samples were subjected to hydrostatic pressure (HP) at 345 MPa for 5 min at 60°C and stored at 4 or 12°C for 84 days or at 25°C for 7days. The HP treatment of roast beef samples inoculated with a mixture of clostridial spores could be stored for 42 days at 4°C. The HP in combination with either BP, or BP, extended the shelf-life of roast beef up to 7 days at 25°C. The combined treatment of HP and BP controlled the growth of *C. laramie* spores and extended the shelf-life of roast beef for 84 days when stored at 4°C.

Films containing antibacterial reagents, ethylenediamine-tetraacetic acid disodium salt (EDTA) and NisaplinVR, were produced by coextrusion with poly (lactic acid) in the presence of a pharmaceutical grade glycerol triacetate. The incorporation of EDTA-NisaplinVR particles resulted in a heterogeneous biphasic structure, as revealed by scanning electronic microscopy, confocal laser microscopy, and acoustic emission tests. The inclusion of glycerol triacetate reduced the Young's modulus and tensile strength, while enhancing the flexibility and the toughness of the resulting blends. The inclusion of the plasticizer also allowed the extrusion to occur at a temperature as low as 120°C to maintain the biological activity of NisaplinVR, which in combination with EDTA, plays a synergistic effect on suppression of the growth of the Gram-negative bacteria, *E. coli* O157:H7. The films thus obtained show potential as packaging materials with a wide spectrum of antimicrobial activity (Liu et al. 2010).

Cronobacter spp. can be found in a variety of foods, including infant milk, milk powder, ultrahigh-temperature–treated milk, cheese products, meat, rice, vegetables, herbs, and spices, and in food production environments Thirty-three antimicrobial agents, including antimicrobial peptides (nisin, lacticin 3147, isracidin), organic acids, emulsifiers (organic acid esters), glycine, lysozyme, tocopherol, EDTA, milk fat globule membrane, and the lactoperoxidase system (LPOS) were screened for anti–Cronobacter sakazakii activity. The compounds were initially screened individually in parallel in synthetic media. Those showing antimicrobial activity were then tested in reconstituted whole milk and finally in reconstituted powdered infant formula (PIF), using mild temperatures of reconstitution and prolonged storage at room temperature. Propionic acid and monocaprylin (as POEM M-100) in combination showed inhibitory activity at sufficiently low concentrations (0.1 to 0.2%) in milk to be considered as potential antimicrobial additives for the inhibition of *C. sakazakii* in reconstituted PIF. More interestingly, LPOS, when combined with the broad-spectrum bacteriocins nisin or lacticin 3147, inhibited outgrowth of *C. sakazakii* at 37°C for 8 h. The combined effects of POEM M-100 and either acetate or propionate and LPOS with lacticin 3147 or nisin were evaluated under the Food and Agriculture Organization of the United Nations–World Health Organization high-risk scenario for PIF, i.e., low temperature of reconstitution and long storage or feeding times at ambient temperature. In the presence of LPOS and lacticin 3147, growth of *Cronobacter* spp. was inhibited for up to 12 h when the PIF was rehydrated at 40 or 50°C. These results highlight the potential of combinatory approaches to improving the safety of infant milk formula (Oshima et al. 2012).

S. aureus and its biofilm formation are recognized as a serious clinical problem. Biofilm formation is also important for survival of staphylococci in the food industry, and several studies have already shown the adhesion capacity of food-related staphylococcal strains. Control of staphylococci during cleaning and disinfection is important to the food industry. Broad-spectrum bacteriocins with proved anti-staphylococcal activity, such as enterocin AS-48, could open new possibilities for disinfection in combination with biocides. In the present study, enterocin AS-48 was tested singly or in combination with biocides against a cocktail of six *Staphylococcus aureus* strains (including three methicillin-resistant strains) in planktonic state as well as in biofilms formed on polystyrene microtiter plates. Cells were challenged with enterocin, biocides or enterocin/biocide combinations. Inactivation of planktonic cells increased significantly (p b 0.05) when enterocin AS-48 (25 mg/l) was tested in combination with benzalkonium chloride (BC), cetrimide (CT) and hexadecylpyridinium chloride (HDP), and non-significantly in combination with didecyldimethylammonium bromide (AB), triclosan (TC), hexachlorophene (CF), polyhexamethylen guanidinium chloride (PHMG), chlorhexidine (CH) or P3-oxonia (OX). In the sessile state (24 h biofilms), staphylococci required higher biocide concentrations in most cases, except for OX. Inactivation of sessile staphylococci increased remarkably when biocides were applied in combination with enterocin AS-48, especially when the bacteriocin was added at 50 mg/l. Results from this study suggest that selected combinations of enterocin AS-48 and biocides offer potential use against planktonic and sessile, methicillin-sensitive and methicillin-resistant *S. aureus* (Caballero Gomez et al. 2013).

Treatment of meat with gamma radiation for inactivation of foodborne pathogens might cause undesirable quality changes in the product. The objective of a study was to use nisin for enhancing the lethality of gamma radiation against *Listeria monocytogenes*, so that moderate doses of radiation can effectively eliminate the pathogen on meat. Cubes of

raw meat (10 g each) were inoculated with *L. monocytogenes* (10^7CFU/g) and treated with nisin (103 IU/g), gamma radiation (0.25 to 1.5 kGy), or combinations of these treatments. Meat was analyzed for *L. monocytogenes* survivors immediately after treatment and during storage at 4°C for up to 72 h. Nisin treatment alone inactivated *L. monocytogenes* by 1.2 log CFU/g. Gamma radiation caused dose-dependent inactivation of the pathogen. Treatment with combinations of nisin and gamma radiation resulted in an additive antimicrobial effect when inoculated meat was tested during the first 24 h and in a synergistic effect when tested after 72 h of storage at 4°C. When *L. monocytogenes* was inoculated onto meat at low levels (4 x 10^3CFU/g), treated with nisin (103 IU/g), and then irradiated (1.5 kGy) and stored at 4uC for 72 h, the pathogen's most probable number was ,0.03/g, indicating that such a combination is potentially effective in eliminating *L. monocytogenes* in meat (Mohamed et al. 2011).

Naghmouchi et al. (2013) performed a study aimed to assess the in vitro activity of colistin alone or in combination with two bacteriocins, nisin A and pediocin PA-1/AcH, against *Salmonella choleraesuis* ATCC 14028, *Pseudomonas aeruginosa* ATCC 27853, *Yersinia enterocolitica* ATCC 9610, and *Escherichia coli* ATCC 35150 (O157:H7). The strain most sensitive to colistin was enterohemorrhagic *E. coli* O157:H7, which was inhibited at a concentration of about 0.12 g/ml. When nisin A (1.70 g/ml) or pediocin PA-1/AcH (1.56 g/ml) was combined with colistin, the concentrations required to inhibit *E. coli* O157:H7 were 0.01 and 0.03 g/ml, respectively. The in vitro antigenotoxic effect of colistin was determined by using the comet assay method to measure the level of DNA damage in freshly isolated human peripheral blood leukocytes (PBLs) incubated with colistin for 1 h at 37°C. Besides the synergistic effect, the combination of colistin (1 mg/ml) and nisin (2 mg/ml) permitted to the researchers to re-evaluate the toxic effect of colistin on Vero (monkey kidney epithelial) cells.

5. Conclusions

The studies conducted to date indicate that interest on bacteriocins will be in continuation increasingly. Along with the growing consumer refusal of chemical additives to combat undesired bacterial growth in foods and beverages, there is a growing demand for alternative antimicrobial treatments and bacteriocins are well accepted natural means of selective microbial inhibition. It is known that most of bacteria are able to produce bacteriocin and each bacteriocin has own specific properties. Thus, all the studies carried on a different bacteriocin are important to show new alternatives in food preservation.

In fact new trends in this fiels indicated that bacteriocin usage in hurdle technology is more effective on pathogen inhibition. Furthermore, it can be concluded that in addition to the traditional hurdle technology represented by low temperature and vacuum packaging or MAP, the exploitation of bacteriocinogenic cultures, as well as their pure bacteriocins holds a great potential for extension of shelf-life and improvement of microbiological safety of vegetable raw materials and final products. Another future aspect of these studies are related to predictive microbiology. Predictive microbiology is frequently applied in the area of food microbiology to develop and apply mathematical models to simulate the responses of undesirable microorganisms to specified environmental variables Recently, there has also been interest in the modeling of beneficial microorganisms deliberately added to food to produce a desired effect. For instance, modeling of the functionality of bacteriocin-producing lactic acid bacteria seems promising for the prediction of bacteriocin bioactivity in foods.

On the other hand, restrictive food legislation for bacteriocins approval and acceptance as food preservatives is the lack point on their usage. Indeed, despite bacteriocins as forerunners in biopreservation technology it is stil surprising that nisin and pediocin PA-1 are commercially available. This problem can be overcome by moving the studies out of the laboratory scale.

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