



Bacteriocin as an advanced technology in food industry.

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Abstract

Concentration in novel biological preservation has increased during recent years, supported by research indicating that antagonistic microorganisms or their antimicrobial metabolites may have some potential as natural preservatives to control the growth of pathogenic bacteria in foods, and also to control mycotoxinogenic fungi. Bacteriocins produced by lactic acid bacteria (LAB) are attracting considerable interest as food preservatives and safe alternatives to conventional antimicrobials in food industry as novel technology. The current global response to these useful bacteriocins needs to be improved by genetic or metabolic engineering. Due to the alarming rise in antibiotic resistance and adverse effects provoked by a number of antibiotics, bacteriocins have been applied in several fields: human health, food industry, animal health, and medicine, in particular as a substitution for the traditional growth promoters, antibiotics. Lactic Acid Bacteria (LAB) bacteriocins are likely used because of their “safe” (GRAS) status, especially in food industry as bio-preservatives. Among these LAB bacteriocins, commercially marketed, nisin groups produced by *Lactococcus lactis* subsp. *lactis* and pediocins produced by *Pediococcus* sp., are the most characterized by their antibacterial property. Several LAB bacteriocins offer potential applications in food preservation, and the use of bacteriocins in the food industry can help to reduce the addition of chemical preservatives as well as the intensity of heat treatments, resulting in foods which are more naturally preserved and richer in organoleptic and nutritional properties. This can be an alternative to satisfy the increasing consumers demands for safe, fresh-tasting, ready-to-eat, minimally-processed foods and also to develop “novel” food products (e.g. less acidic, or with a lower salt content). Therefore, this review paper is intention to discuss the novel and attractive application of bacteriocin producing by Lactic acid bacteria as innovative technology in food industry.

Keywords: Bacteriocin, food industry, bio-preservative, LAB, novel technology

1. Introduction

Bacteriocins are antimicrobial compounds which are synthesized ribosomally by many members of Lactic Acid Bacteria (LAB) (Garneau et al., 2002). Bacteriocins produced by LAB usually show antibacterial activity against strains closely related to the producers as well as Gram-positive food spoilage and food-poisoning bacteria such as *Bacillus* spp., *Clostridium* spp., *Staphylococcus* spp., and *Listeria* spp. LAB bacteriocins are typically tolerant of high temperatures and low pH, and are degradable by digestion enzyme. In addition to these characteristics,

since LAB are beneficial and generally regarded as safe, LAB bacteriocins have attracted special interest for further applications, e.g., food preservatives. In fact, nisin A, the best studied bacteriocin, produced by certain strains of *Lactococcus lactis*, is used as a food preservative in more than 50 countries. In addition to nisin A, various LAB bacteriocins have been discovered and characterized. On the basis of the food-grade safety of LAB bacteriocins, they are expected to be safe antimicrobial agents and food preservatives. In addition, no side effects and no development of

resistant bacteria have been reported in the practical use of LAB bacteriocins. One reason is that they act through quick pore formation on the target cell membrane at extremely low concentrations. Another is the proteinaceous nature of LAB bacteriocins, which enable them to be degraded easily in the human body and in the environment. Various bacteriocins have been identified in Gram positive bacteria, including LAB. Among the various bacteriocins from LAB, nisin A has been studied extensively in its uses for food preservation and as a safer alternative to conventional antimicrobials (Cleveland *et al.*, 2001 and Nishie *et al.*, 2012). Some LAB bacteriocins have been found to have higher activity against certain food-poisoning bacteria than nisin A (Ennahar *et al.*, 2000 and Drider *et al.*, 2006).

Bacteriocins have long attracted the interest of food sector as potential natural food preservatives against spoilage and pathogenic bacteria (Kumar *et al.* 2011). Nisin, pediocin and other bacteriocins produced by lactic acid bacteria (LAB) have received a great deal of attention because of their beneficial effects to human health and to food production as well as the replacement of chemical preservatives that are being continuously questioned with regard of safety (Zendo 2013). Thus, this potential offers a logical explanation for the expanding trend of applications of LAB in the food industry (Papagianni and Anastasiadou 2009). In addition, no side effects and no development of resistant bacteria have been reported in the practical use of LAB bacteriocins (Zendo 2013). The administration of bacteriocin-producing bacteria rather than the bacteriocins themselves might be a more cost-effective approach, but significant progress in developing suitable producer strains will have to be made before such an approach will be feasible (Joerger 2003). In order to achieve improved food safety against such pathogens, food industry makes use of chemical preservatives or physical treatments (e.g. high temperatures). These preservation techniques have many drawbacks which includes the proven toxicity of the 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) or their metabolites, Bacteriocin (Nath *et al.*, 2013). The increasing demand for safe food has

increased the interest in replacing chemical additives by natural products, without injuring the host or the environment. Biotechnology in the food-processing sector targets the selection, production and improvement of useful microorganisms and their products, as well as their technical application in food quality. The use of non-pathogenic microorganisms and/or their metabolites to improve microbiological safety and extend the shelf life of foods is defined as bio preservation (Martinis *et al.*, 2001). Antagonistic properties of LAB allied to their safe history of use in traditional fermented food products make them very attractive to be used as bio preservatives (Caplice and Fitzgerald, 1999). In this review, current trends and perspectives on the applications LAB bacteriocins in food industry as innovative technology as bio-preservatives are discuss in detail.

Lactic acid bacteria

The LAB group is currently classified in the phylum Firmicutes, class Bacilli, and order Latobacillales. LAB are classified based on cellular morphology, mode of glucose fermentation, range of growth temperature, and sugar utilization patterns (Quinto, *et al.*, 2014). In addition, sequencing of 16S rRNA gene is an important guideline in the classification of LAB (Wood &Holzapfel, 2012). LAB genera include *Lactobacillus*, *Lactococcus*, *Leuconostoc*, *Pediococcus*, *Streptococcus*, *Aerococcus*, *Alloiococcus*, *Carnobacterium*, *Dolosigranulum*, *Enterococcus*, *Oenococcus*, *Tetragenococcus*, *Vagococcus* and *Weissella* (Khalid, 2011 and Walter 200), with *Lactobacillus* being the largest genus, including more than 100 species that are abundant in carbohydrate-rich substances.

Due to their health benefits, some LAB are used as probiotics, biological food preservatives and biological control agents against food borne pathogens and infectious diseases (Smaoui *et al.*, 2010). Lactic acid bacteria (LAB) have been used for bio preservation in several foods. This bio preservation ability is due to production of several broad-spectrum antimicrobial compounds and most of the LAB have generally regarded as safe (GRAS) status. The antimicrobial effect of LAB is mainly related to the production of lactic- and acetic-acids, as well as propionic-, sorbic-, benzoic-acids, hydrogen peroxide, diacetyl, ethanol, phenolic- and proteinaceous-compounds however some strains are able to synthesize antimicrobial substances e bacteriocins (Dalié *et al.*, 2010). Bacteriocins from the generally recognized as safe LAB, have received significant

attention as a novel approach to the control of pathogens in foods (Settani et al., 2005). The prevention of foods by natural and microbiological compounds 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 (Galvez et al., 2008).

Bacteriocin produced by LAB

Bacteriocins are peptides or complex proteins biologically active with antimicrobial action against other bacteria, principally closely related species (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 and Saavedra et al., 2004). Bacteriocin production could be considered as an advantage for food and feed producers since, in sufficient amounts, these peptides can kill or inhibit pathogenic bacteria that compete for the same ecological niche or nutrient pool. This role is supported by the fact that many bacteriocins have a narrow host range, and is likely to be most effective against related bacteria with nutritive demands for the same scarce resources (Deegan et al., 2006). Most of the new bacteriocins belong to the class II bacteriocins which are small (30–100 amino acids) heat-stable and commonly not posttranslational modified. While most bacteriocin producers synthesize only one bacteriocin, it has been shown that several LAB produce multiple bacteriocins (2–3 bacteriocins). 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).

Classification of Bacteriocin

According to Klaenhammer (1993), bacteriocins can be divided into four classes. The class I of lantibiotics, represented by nisin, gathers very low molecular weight (<5 kDa) thermostable peptides characterized by the presence of lanthionine and derivatives. The class II is composed of small thermostable peptides (<10 kDa) divided into three subclasses: IIa (pediocin and enterocin), IIb (lactocin G) and IIc (lactocin B). The class III is represented by

highmolecular weight (>30 kDa) thermolabile peptides such as thehelveticin J, while in the class IV we can find large peptides complexes with carbohydrates or lipids. However, Cleveland et al. (2001) believe that these structures are artifacts of partial purification and not a new class of bacteriocins. Cotter et al. (2005) suggested a new classification where bacteriocins are divided into two categories: lantibiotics (class I) and not containing lanthionine lantibiotics (class II), while high molecular weight thermolabile peptides, which are formally components of the above class III, would be separately designated as “bacteriolysins”. These authors also suggested that the above class IV should be extinguished. Finally, Drider, et al. (2006) divided bacteriocins into three major classes according to their genetic and biochemical characteristics and we will refer to such a classification in the following one by one.

Class I

Lantibiotics are small peptides with rare thermostable amino acids in their composition, which may result from the combination of two alanine linked by a disulfide bond as for lanthionine, or from an amino butyric acid linked to an alanine by a disulfide bond as for b-methyl-lanthionine (Jarvis., 1968). The main representative of this class is nisin, which is produced by some strains of *Lactococcus lactis* subsp. *lactis* and is composed of 34 amino acid residues. Toxicological studies showed that nisin intake does not cause any toxic effect to humans with an estimated lethal dose (LD50) as high as 6950 mg/kg (close to that of salt) when administered orally (Jozala et al., 2007). In general, some authors have ascribed the high LD50 values of bacteriocins to digestive enzymes capable of rapidly inactivating trypsin and chymotrypsin produced in the pancreas (Vaucher et al., 2011). Nisin has been largely using in the food industry as antibotulinic agent in cheese and liquid eggs, sauces and canned foods. It exhibits a wide-spectrum antimicrobial action against *L. monocytogenes*, *Staphylococcus aureus*, *Bacillus cereus* and other pathogens and LAB species (Rilla et al. 2004), which is mediated by a dual action mechanism encompassing interference with cell wall synthesis and promotion of pore formation in cell membrane. Nisin is the only bacteriocin approved for food applications being considered to be safe by the Food and Agriculture Organization/World Health Organization (FAO/WHO) in 1969. In addition, it was also included as biopreservative ingredient in the European food additive list, where it was assigned the number E234.

Class II

This subclass is composed of small thermostable peptides (<10 kDa) with an amphiphilic helical structure that allows for their insertion in the cytoplasmic membrane of the target cell, thereby promoting membrane depolarization and cell death and there are three subdivisions to Drider et al. (2006).

Subclass IIA

The subclass IIA is composed of bacteriocins showing high specificity against *L. monocytogenes*. Its representatives have 37-48 amino acid residues with an N-terminal portion with pleated sheet configuration and a C terminus containing one or two α -helices (Fimland et al., 2005). Pediocin PA-1, which is composed of 44 amino acid residues, is the only bacteriocin belonging to the subclass IIA that is synthesized not only by different species, but also by different genera of LAB. It was initially detected in *Pediococcus acidilactici* and since then, other strains and species of pediococci were described as producers of pediocin (Díez et al., 2012). The first enterocin was identified by Kjems (1955) and subsequently classified as a member of the pediocin family. Since then, several enterocins have been described, that have representatives in more than one class of bacteriocins.

Subclass IIB

This subclass includes heterodimeric bacteriocins, i.e. bacteriocins that require the combined activity of two peptides. Normally, genes are located in the same operon and expressed simultaneously, and the two peptides act in combination frequently showing an important synergistic action. Their mechanism of action also involves the dissipation of membrane potential and a decrease in the intracellular ATP concentration. These peptides have very low activity when individually employed (Garneau et al., 2002).

Subclass IIC

Bacteriocins belonging to this subclass have a covalent bond between C and N terminals, resulting in a cyclic structure (Kawai et al., 2004). Enterocin AS-48, circularin A and reuterin 6 are representatives of this subclass.

Class III

This class gathers large thermolabile bacteriocins (>30 kDa) that have complex activity and protein structure.

Their action mechanism is different from those of other bacteriocins, in that they promote lysis of the cell wall of the target microorganism. Their N-terminal portion is homologous to an endopeptidase involved in cell wall synthesis, while the C-terminal portion is responsible for recognition of the target cell (Lai et al., 2002).

Class IV

It is presently reserved for cyclic bacteriocins composed not only from protein (also lipid or carbohydrate) (Klaenhammer, 1993). Class IV of complex bacteriocins that require non-proteinaceous moieties like carbohydrate or lipid for their activity has also been suggested by some authors, however, bacteriocins in this class have not been characterized convincingly, and hence definition of this class requires additional characterization.

Bio-preservation

The use of non-pathogenic microorganisms or their metabolites to improve microbiological safety and extend the shelf life of foods is defined as bio-preservation (Nath et al., 2013 and Martinis et al., 2001). Bio-preservation refers to extended storage life and enhanced safety of foods using the natural microflora and (or) their antibacterial products. It 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 (Martinis et al., 2001 and Stiles, 1996). One of the most common forms of food bio-preservation is fermentation, a process based on the growth of microorganisms in foods, whether natural or added. It employs the breakdown of complex compounds, production of acids and alcohols, synthesis of Vitamin-B12, riboflavin and Vitamin-C precursor, ensures antifungal activity and improvement of organoleptic qualities such as, production of flavor and aroma compounds. In fish processing, bio-preservation is achieved by adding antimicrobials or by increasing the acidity of the fish muscle. Efforts have concentrated on identification and development of protective bacterial cultures with antimicrobial effects against known pathogens and spoilage organisms. Following compounds such as organic acids, bacteriocins, diacetyl and acetaldehyde, enzymes, CO₂, hydrogen peroxide etc. are contributing to antimicrobial activity by Microbiota.

Bacteriocin as bio-preservative in foods industry

Before bacteriocin can be applied in foods their cytolytic abilities should be assessed in detail. This is a very important issue since recently a cytolysin produced by *E. faecalis* was described that possesses both haemolytic and bacteriocin activities (Gillmore et al., 1990). Recombinant DNA technology is currently applied, to enhance production, to transfer bacteriocin genes to other species and for mutation and selection of bacteriocin variants with increased and/or broad activity spectra (Osmanagaoglu et al., 2001). Continued study of the physical and chemical properties, mode of action and structure-function relationships of bacteriocins is necessary if their potential in food preservation is to be exploited. Further research into the synergistic reactions of these compounds and other natural preservatives, in combination with advanced technologies could result in replacement of chemical preservatives, or could allow less severe processing (eg. heat) treatments, while still maintaining adequate microbiological safety and quality in foods.

Biological preservation approaches seem attractive as a safety parameter in foods with reduced contents of ingredients such as salt, sugar, fat and acid that usually serve as factors potentially inhibitory to microbial growth. It is expected that biological preservation methods may enjoy better consumer acceptance than their preservation counterparts that use traditional chemical preservatives. Bacteriocin-producing LAB has potential for the preservation of foods of plant origin, especially minimally processed vegetables, such as prepackaged mixed salads and fermented ones. Vescovo et al (1995) observed a reduction in high initial bacterial loads of ready-to-use mixed salads on addition of bacteriocin-producing LAB. Furthermore, bacteriocin-producing starter cultures may be useful for fermentation of sauerkraut or olives to prevent the growth of spoilage organisms. Nisin is suitable for use in a wide range of foods liquid or solid, canned or packaged, chill or warm ambient storage and it is best added as an aqueous solution, usually to the liquid portion of a product during its processing. It can also be added as a powder, but in all cases, it is essential to ensure uniform dispersal throughout the food matrix. The level of nisin addition depends on the type of food, severity of heat process, storage conditions and the required shelf-life. Nisin is often used in acidic foods, but is effective in products across a wide range of pH values (3.5-8.0). It seems to be a very effective preservative in liquid egg, which generally has a pH of 7.3 to 7.8. It is used in a variety of products including

pasteurized, flavored and long life milks, aged and processed cheeses, and canned vegetables and soups. Nisin has been utilized to inhibit undesirable LAB in wine and beer (Schillinger et al., 1996; Mulet et al., 1998; Daesche et al., 1991; Ogden et al., 1998 and Srivatsa et al., 1994).

Very few studies have been reported on the applications of pediocins, other than a few challenge studies against spoilage and pathogenic bacteria in several food systems. Pediocin PA-1/AcH, besides nisin, is the most studied bacteriocin of LAB. Pediocin PA-1/AcH has been demonstrated to effectively reduce populations of *Listeria* strains in ice cream mix, sausage mix, fresh and ground beef and whole milk. It has been found to be effective against many strains of sub-lethally stressed Gram positive and Gram-negative spoilage and pathogenic bacteria. Such injured bacteria can be present in foods that have a pH below 6, water activity below 0.9, or have been given low heat treatment, subjected to hydrostatic pressure, or stored at low temperature, including long-term storage at refrigerated temperature (for mesophilic and thermophilic bacteria). Incorporation of pediocins as preservatives in such foods can help in killing the normally sensitive and resistant but injured cells of spoilage and pathogenic bacteria and ensure longer product shelf-life and greater consumer safety. In the production of certain fermented foods, especially in controlled fermentation where specific strains of starter cultures are used, pediocin PA-1/AcH has a specific application to control *L. monocytogenes*. Currently of interest is the use of nisin with novel preservation techniques such as ultra-high pressure or high hydrostatic pressure and pulsed electric field. Future approaches should consider the application of bacteriocins in combination with treatments enhancing their effectiveness in foods. The antimicrobial efficiency of a bacteriocin may also be enhanced or broadened by using it in combination with other bacteriocins or other compounds including surfactants, chelating agents or other metal completing compounds (Bennik et al., 1997; Ennahar et al., 1998 and Bhunia et al., 1990).

Bioengineering of Bacteriocins

In last two decades, there have been significant advances in functional genomic analysis of LAB and their biochemical characterization of bacteriocins. Considerable efforts have been made to functionally characterize bacteriocin operons and to express them in heterologous systems (Osmanagaoglu et al. 2000

and Tominaga and Hatakeyama 2007). The genes responsible for bacteriocin production are frequently associated with mobilisable elements, or in the chromosome in association with transposons or plasmids (Belkum et al. 1998). The low-molecular-weight bacteriocins of Gram-positive bacteria generally appear to be translated as pre-peptides that are subsequently modified to form the mature biologically active (bactericidal) molecules (Buchman et al. 1998). Specific auxiliary functions required by bacteriocin-producing cells include mechanisms for extracellular translocation of the bacteriocin and for self-immunity to the bactericidal activity of the molecule (Jack et al. 1995). As is the case for most bacteriocins, the lantibiotics are initially synthesized with an N-terminal leader peptide. In general, the pre-peptide is modified by the action of other proteins encoded by the bacteriocin gene cluster before export (Deegan et al. 2006).

Combination of bacteriocins with chemical substances and natural Antimicrobials

Combination of bacteriocins with chemical substances and natural Antimicrobials Presence of NaCl enhanced the antimicrobial action of bacteriocins such as nisin, leucocin F10, enterocin AS-48 and others (Ananou et al., 2004). Sodium chloride may also induce conformational changes of bacteriocins (Lee et al., 1993) or changes in the cell envelope of the target organisms (Jydegaard et al., 2000). The combinations of nisin and nitrite delayed botulinal toxin formation in meat systems and showed increased activity on clostridial endospores outgrowth and also on *Leuconostoc mesenteroides* and *L. monocytogenes* (Gill and Holley, 2003). Addition of nitrite also increased the anti-listeria activity of bacteriocinogenic lactobacilli in meat and the activities of enterocin EJ97 against *L. monocytogenes*, *Bacillus coagulans* and *Bacillus macroides* (Garcia et al., 2004)

The enhanced effect of chelators such as EDTA, disodium pyrophosphate, trisodium phosphate, hexameta phosphate or citrate and bacteriocins against Gram-negative bacteria has been demonstrated for nisin both under laboratory conditions and in foods (Fang and Tsai, 2003). Other antimicrobial compounds such as ethanol can act synergistically with nisin to reduce the survival of *L. monocytogenes* (Brewer et al., 2002). Essential oils and their active components, the phenolic compounds are also attractive natural preservatives. When used in combination with bacteriocins, the dose of added phenolic compounds could be lowered thereby decreasing their

impact on the food flavour and taste. Nisin acted synergistically with carvacrol, eugenol or thymol against *B. cereus* and/or *L. monocytogenes*. Combinations of nisin with carvacrol, eugenol, or thymol resulted in synergistic action against *Bacillus subtilis* and *Listeria innocua*, while nisin and cinnamic acid had synergistic activity against *L. innocua*, but only additive against *B. subtilis*. Carvacrol (0.5 mM) was used to enhance the synergy found between nisin and a pulsed electric field treatment (PEF) against vegetative cells of *B. cereus* in milk (Pol et al., 2001). The combination of nisin and cinnamon accelerates death of *Salmonella Typhimurium* and *Escherichia coli* O157:H7 in apple juice (Grande et al., 2007)

Purification of LAB bacteriocins

Most of purification techniques initiate with separation of cell free supernatant then concentration of culture supernatant like in salt precipitation (e.g. extraction with organic solvents, ammonium sulphate, acid precipitation (Muriana and Luchansky, 1993) adsorption of bacteriocins onto the producing cells at pH 5.5-6.8 or hydrophobic matrix such as amberlite XAD-16 (Cintas *et al.*, 2000). Subsequently, several chromatographic steps including size exclusion (gel filtration), adsorption (ion-exchange), or hydrophobic interaction (reverse-phase) chromatography have been used to achieve significant purification of bacteriocins (Wu *et al.*, 2004). Todorov *et al.*, (2004) summarized the purification method used by others researchers as follow: (1) ammonium sulphate precipitation, and cation exchange-SP-sepharose, reversed-stationary-phase (octyl-sepharose-CL-4B), stationary-phase C2/C18 chromatography. (2) anion-exchange chromatography (DEAE-Sephadex A-25) and reverse-phase HPLC cation-exchange chromatography (SP-sepharose fast-flow cation exchange column), C2/C18 reverse-phase chromatography and hydrophobic interaction chromatography (phenyl-sepharose CL-4B column) (3) ammonium sulphate precipitation (40%), and cation exchange-SP-sepharose (4) ammonium sulphate precipitation (55%), hydrophobic interaction (C8), cation exchange chromatography Mono S cation exchange column (pharmacia, Biotech). (5) ammonium sulphate precipitation (80%).

Characterization of bacteriocins

Maximum LAB bacteriocins are hydrophobic, amphiphilic molecules and cationic composed of twenty to sixty residues of amino acid (Chen and Hoover, 2003). In addition, Oscariz and Pisabarro

(2001) supported that cationic and highly hydrophobic are two basic principles which almost maximum of bacteriocins should fulfill. Maximum bacteriocins which are small in size are dynamic towards broad pH range i.e. 3.0-9.0 and while at extreme pH resistance is shown at 11.0 (bavaricin A), 1.0 (acidocin B) and has been observed, at pH 7.0 maximum of these bacteriocins are cationic e.g lactocin S which have net charge of -1 at neutral pH being the exception. Bacteriocins have higher isoelectric point which permits them to interact at physiological pH level with the bacterial membranes surface which is anionic. This interaction can be sufficient in the case of wide inhibitory spectrum bacteriocins and make possible in the case of receptors requiring compound addition of the hydrophobic moiety into the membrane of bacteria. A physical, rapid and sensitive method for detection of bacteriocins could be a functional to track purification actions to identify production of bacteriocin during experiments concerning genetic exploitation and to identify bacteriocins in food.

In culture or food products presence of bacteriocins is confirmed by method of searching appropriate molecular weight compounds. Matrix assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) appears to have potential as one such method (Rose *et al.*, 1999). For determination of masses of purified and partially purified bacteriocins of class I and II and effective for proteins and peptides of molecular masses in range of 0.5-30 kDa is determined by MALDI-TOF MS (Hindre *et al.*, 2003). In addition, molecular weight of SA-FF22 bacteriocin like substance was analyzed by ESI-MS (Jack *et al.*, 1996). Moreover, an extra ordinary powerful tool for determination of biological samples' molecular mass is electrospray-ionization mass spectrometry (ESI-MS) (Walk *et al.*, 1999).

The last most important data required for complete characterization are their sequence of amino acids is determined by using automated protein sequencer by Edman degradation (Jack *et al.*, 1996). However, Walk *et al.* (1999) reported that during automated protein sequencing numerous troubles might arise of compounds containing nonproteinogenic amino acids. Complete amino acid sequence was determined by using the coupled sequenator-ESI-MS system. To get a complete sequence of bacteriocin, the DNA sequence encoding the bacteriocin can be analyzed (Zendo *et al.*, 2003). Moreover, studies should be focused on gene cluster analyses which are taking part in immunity and production of bacteriocins (Folli *et al.*, 2003).

Biosynthesis of bacteriocins

The bacteriocins production is normally associated with growth due to the reason that most of production occurs for the period of middle of exponential phase and at end of exponential phase, it boost up to a maximum level till establishment of the early-stationary phase (Cheigh *et al.*, 2002). In addition, other reports supported that this loss in bacteriocin activity may be due to degradation by endogenous protease induced during the growth phase and/or the adsorption of bacteriocin on the surface of the producer (Onda *et al.*, 2003). Genes coding for active bacteriocins are usually in operon clusters (Cleveland *et al.*, 2001). The genes encoding bacteriocin production and immunity are usually organized in operon clusters (McAuliffe *et al.*, 2001). Normally at least four different genes are necessary to accomplish the bacteriocins production of LAB: (i) a devoted immunity gene (ii) a prepeptide structural encoding of gene (iii) a transporter ABC- encoding is dedicated and (iv) a gene encoding the secretion machinery (Garneau *et al.*, 2002; Chen and Hoover, 2003). Bacteriocin production genes encoding can be located on the chromosome or encoded in a plasmid or transposon (Chen and Hoover, 2003).

In Bacteriocins synthesis, first prepeptide and precursor which are biologically inactive and have an N-terminal expansion sequence of leader. Subsequence breakage of this prepeptide at a particular processing site breaks the leader sequence from the antimicrobial molecule concurrently with its expulsion to the outside of the cell. The mature bacteriocin's translocation through cytoplasmic membrane is mediated by ABC-transporter and accessory protein. The 3-component regulatory system typically includes histidine proteinase (HPK), response regulator (RR) and induction factor (IF), that is needed for indication to induce the transcription of target genes. The immunity proteins provide total immunity towards the bacteriocin producer's strains (Eijsink *et al.*, 2002).

Commercial Production of Bacteriocins

Several bacteriocin-producing bacteria have been patented, but to the end of 2005 none of them were at the commercialization stage (Doyle *et al.* 1999; Shotts and Wooley 2000), the only commercially produced bacteriocins are the group of nisin produced by *Lactococcus lactis* (Jones *et al.* 2005) and pediocin PA-1 by *Pediococcus acidilactici* (Gálvez *et al.* 2008). Nisin is the most commercially important member of a

large class of bacteriocins produced by bacteria that can kill or inhibit the growth of other bacteria. This phase of the Nisin Market Study analyzes the characteristics of the current market for nisin and competing bacteriocins in four main sections highlighting: (1) the general market characteristics for antimicrobial preservatives; (2) current producers and sellers of commercial grade nisin; (3) current users of nisin and competing bacteriocins; and (4) implications for the market opportunities for nisin production in the U.S. The global leader in the antimicrobial preservatives industry is Danisco A/S, a Danish company, with Royal DSM (Netherlands), and Kerry Bio-Sciences (Ireland) considered being their peer competitors in the bio-preservatives sector (Jones et al. 2005). Danisco's Nisaplin™ is generally considered to be the most commercially available form of nisin for food preservative uses. Danisco's strategic focus for their nisin product line is the U.S. meat and deli food sector in order to take advantage of the FDA approval status of nisin as a natural ingredient. Other players in the global nisin market include Rhodia, S.A. (France) along with numerous producers and providers of various antimicrobial products based in China. Some of these Chinese sources are in joint ventures or alliances with European-based corporate entities.

Bacteriocin preservatives are part of the \$22 billion global food additives market that has grown at 2-3% per annum through 2007 to \$24 billion. The Genencor division of Danisco has manufacturing locations in the United States, Finland, Belgium, China, and Argentina (<http://www.genencor.com>). More than half of Genencor's \$410 million yearly sales are outside the United States (Law 2005). Several attempts have also been tried to express and secrete pediocin PA-1 in other *L. lactis* hosts, resulting in the enhanced production of pediocin PA-1 and to coproduce the antibiotic nisin A and pediocin PA-1 and develop novel expression system for large-scale production and purification of recombinant class IIa bacteriocins and its application to Piscicolin (Gibbs et al. 2004). More recently the bacteriocin sakacin A (SakA) and two SakA-derived expressed as chimeras in lactic acid bacteria (LAB) and the yeast *Pichia pastoris* and *Kluyveromyces lactis* (Jiménez et al. 2013).

Potential application of LAB and bacteriocins in the food industry

Many LAB used in the food industry produce bacteriocins and thus are a rich source of these natural inhibitors. These bacteriocins have potential applications in preservation for improving the safety

and quality of foods. For example, nisin produced by several strains of *Lactococcus lactis* is the best known, most well characterized and the only antibiotic to have realized widespread commercial use. *Lactobacillus plantarum* produces several bacteriocins like plantaricin 35d, bacteriocins ST28MS and ST26MS that are active against the pathogens and spoilage microorganisms in foods (Todorov et al., 2004 and 2005). Another bacteriocin has potential to be used in the food industry is pediocin which is an antilisterial bacteriocin produced by several *Pediococcus* strains. Up to now, different strategies have been used to incorporate bacteriocins into foods. They can be produced by live cultures directly in fermented food by using bacteriocin producing starter cultures or they can be applied to the food as a protective culture by spraying them onto the surface of cheese (O'Sullivan et al., 2006). Bacteriocins can be added directly to the food as an additive, such as the enriched nisin product, Nisaplin®. They may also be included in packaging materials such as nisin coatings on low density polyethylene film or barrier films with methyl cellulose (Cooksey, 2005).

Nisin is commercially available as a powder called Nisaplin® and Novasin™ (Danisco), which contains the active ingredient of 2.5% nisin, along with 77.5% NaCl and non-fat dry milk (12% protein and 6% carbohydrate). Nisin has a broad spectrum of antimicrobial activity against gram-positive bacteria and has been shown to be bactericidal to *Staphylococcus aureus*, *Listeria monocytogenes*, vegetative cells of *Bacillus* spp. and *Clostridium* spp. as well as preventing outgrowth of spores in *Bacillus* and *Clostridium* species (Klaenhammer et al., 1993). In canned foods, there are two commonly associated spoilage organisms, *B. stearothermophilus* and *C. thermosaccharolyticum*. Addition of nisin in the range of 100-200 IU g⁻¹ controlled these pathogens and positively contributed to overall nutritional and organoleptic properties of the canned product (De Vuyst et al., 1994). Nisin was also used in high acid canned foods such as in canned tomato and fruit juices to control *Clostridium pasteurianum* and *Bacillus* spp. (Delves et al., 1990).

Combinations of nisin with other preservative regimes were also the most effective method of preservation and extension of shelf life for foods. For example, treatment of caviar with nisin (500 IU mL⁻¹) reduced the *L. monocytogenes* numbers by 2.5 logs, while when used in combination with heat at 60 °C for 3 min the pathogen was no longer detected after storage of 28 days at 4 °C (Al-Holy et al 2005). Ukuku et al. (2005)

reported that melons were washed in a solution containing nisin (25µg mL⁻¹), hydrogen peroxide (1%), sodium lactate (1%) and citric acid (0.5%) and population of *E.coli* O157:H7 or *L. monocytogenes* was reduced from 5.27 and 4.07 log cfu cm⁻² to less than 1 log cfu cm⁻². This treatment reduced transfer of bacterial pathogens from whole melon surfaces to fresh-cut pieces.

As biopreservation of fresh and/or fresh-cut products using bacteriocinogenic LAB isolated from vegetables also presents an innovative approach to improve the safety and quality of the products. We applied *Ent. faecium* (13.2) and *Lact. lactis* (7.17) onto fresh-cut salads to investigate their effect on the growth of the natural microflora and *Li.innocua* inoculated on salads. Results showed that the addition of the LAB significantly reduced the loads of natural occurring *Listeria* spp. ($p= 0.033$), yeasts ($p = 0.011$), *Pseudomonas* spp. ($p = 0.010$) and coliforms ($p = 0.011$). *Ent.faecium* and *Lact. lactis* also significantly reduced growth of *Li. innocua* inoculated on fresh-cut salads ($p = 0.005$) during a 10 days storage at 5 °C. Scanning electron microscopy (SEM) revealed a significantly reduced presence of *Li.innocua* on the surface of fresh-cut salads after addition of the bacteriocinogenic LAB (Sharpe, 2009). The bacteriocin producing LAB have the potential to be used as protective cultures in fresh-cut produce to control spoilage and pathogenic microorganisms thereby improving the product shelf-life and safety. Although knowledge about bacteriocins has increased greatly, there are still many questions regarding immunity (self-protection) and the molecular basis of target-cell specificity. Use of genetic methods for the construction and improvement of bacteriocin-producing LAB provides great promise for the food fermentation and preservation industries. Genetic strategies are forthcoming that will direct genes for bacteriocin production and immunity into the desired industrial strains (Klaenhammer, 1998). Further characterization of the bacteriocins and their mechanism of action may result in a better understanding of their implications in food systems. However, applications of bacteriocins for food protection should not substitute good manufacturing practice.

Conclusion and Future prospects for the use of bacteriocins as bio preservatives

A large number of bacteriocins from LAB have been characterized to date, and many different studies have indicated the potential usefulness of

bacteriocins in food preservation. Since the efficacy of bacteriocins in foods is dictated by environmental factors, there is a need to determine more precisely the most effective conditions for application of each particular bacteriocin. However, the combined application of many other technologies (such as ultrasonication, irradiation, microwave and ohmic heating, or pulsed light) still remains unexplored. Bacteriocinogenic cells may also act as living factories in foods. The antimicrobial effects of bacteriocins and bacteriocinogenic cultures in food ecosystems must be understood in terms of microbial interactions.

The particular approach of biological preservation that needs to be taken will be determined by the type of product and the intrinsic and extrinsic parameters existing during processing, storage and distribution. In our opinion, bacteriocin production in situ by starter cultures has a good chance of finding applications in fermented foods. In particular, an additional safety factor seems desirable for mild (low-acid) fermented foods. In non-fermented refrigerated products, such as minimally processed meats or prepacked vegetable salads, only those strains producing sufficient and potent amounts of bacteriocin but no other metabolic compounds at amounts that are detrimental to the sensory quality of the product can be applied. The direct addition of purified bacteriocins obviously provides a more controllable preservative tool in such products. However, the cost factor at present makes this approach unattractive. In general, biological preservation approaches seem attractive as a safety parameter in foods with reduced contents of ingredients such as salt, sugar, fat and acid that usually serve as factors that are potentially inhibitory to microbial growth. It is expected that biological preservation methods may enjoy better consumer acceptance than preservation methods that use traditional chemical preservatives.

Novel LAB bacteriocins and their biosynthetic mechanisms will be useful in applications such as food preservation and peptide design. However, more details of their action mechanisms and biosynthetic mechanisms must be determined for further application. On the other hand, screening too must be continued to discover further novel bacteriocins. This should help in the control of undesirable bacteria and in designing more powerful and more selective antimicrobial peptides.

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