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Biological preservation of foods–Bacteriocins of lactic acid bacteria

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Lactic acid bacteria and their antimicrobial metabolites have potential as natural preservatives to control the growth of spoilage and pathogenic bacteria in foods. To date, nisin is the only bacteriocin that has found practical applications in some industrially processed foods. Its antibacterial activity and possible use as a biopreservative has been studied in a large number of food systems. Its application for the control of some pathogens and food spoilage organisms has been approved in a number of countries. Limited studies have shown that pediocins from several *Pediococcus* strains can also be used effectively in food systems to control *Listeria monocytogenes*. This review discusses the potential of lactic acid bacteria and their principal antimicrobial peptides, bacteriocins in biological preservation of foods. It is anticipated that the advances in bacteriocins research and combination treatments for food preservation will benefit both the consumer and the producer.

Keywords: biological preservation, lactic acid bacteria, bacteriocins, food-borne pathogens, food spoilage bacteria, biopreservatives, nisin, pediocin

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Introduction

In the production of food, it is crucial to take proper measures for ensuring its safety and stability during the shelf-life. Food preservation is carried out to maintain the quality of raw material and physico-chemical properties as well as functional quality of the product whilst providing safe and stable products. In general, the preservation processes consist of a combination of mild heat stress and low concentration of chemical preservatives to control food spoilage and the outgrowth of pathogenic spore-forming bacteria. However, the resistance of some microorganisms to most commonly used preservatives has created problems for the food industry. In particular, modern consumer trends and food legislation have left the successful attainment of this objective to be more of a challenge. Firstly, consumers demand high quality, preservative-free, safe but minimally processed foods with extended shelf life. Secondly, legislation has restricted the use as well as the permitted levels of some of the currently approved preservatives in different foods. These consumer and legislative needs call for innovative approaches to preserve foods[1,2].

Preservation aims at either to eliminate or reduce the outgrowth potential of spoilage and pathogenic organisms in foods. Lesser the severity of preservative treatment, lower is the product damage, meeting both the food industry as well as consumer interests for high quality products with improved organoleptic and nutritional quality while maintaining microbial safety. Much research has been carried out on the antimicrobial effect of heat in combination with modified environmental factors such as low water activity, *p*H[3] and ultra-high pressure[4], but very little has been reported on the combination of physical treatments with natural antimicrobials. Processing at high temperatures is often detrimental to the product quality as it causes a significant reduction in nutritional value and lowers organoleptic quality[5]. Therefore, less severe heat application during processing is desirable.

In spite of the introduction of modern technologies and safety concepts, the reported number of food-borne illnesses and intoxications are on the rise. Many of the ready-to-eat and novel food products represent new food systems with respect to health and spoilage risks. In the light of the above as well as improved understanding and knowledge of the complex microbial interactions, there is an increased tendency to use biopreservatives in the form of protective cultures or their metabolites, i.e., enzymes and bacteriocins[6].

Biological preservation implies a novel scientific approach to improve the microbial safety of foods. By definition, this concept refers to the use of

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antagonistic microorganisms or their metabolic products to inhibit or destroy undesirable microorganisms in foods. Lactic acid bacteria (LAB) have been exploited for thousands of years for the production of fermented foods due to their ability to produce desirable changes in the taste, flavour and texture as well as inhibit pathogenic and spoilage microorganisms. Since they are prevalent in numerous fermented foods, it is assumed that most representatives of this group do not pose any health risk to man, and are designated as GRAS (Generally Recognized as Safe) organisms. The LAB, generally considered as 'food grade' organisms, has a special promise as protective cultures. There are many potential applications of protective cultures in various food systems[6]. The biological preservation of foods may contribute to their health benefits (e.g. *acidophilus* milk). Such probiotic cultures have been considered to be safe and provide substantial health benefits to man[7]. A number of different factors have been identified to contribute to the antimicrobial activity of LAB. These bacteria produce different antimicrobials, such as lactic acid, acetic acid, hydrogen peroxide, carbon dioxide and bacteriocins, which can inhibit pathogenic and spoilage microorganisms, extending the shelf-life and enhancing the safety of food products[6,8]. Bacteriocins and their potential role as novel food preservatives have recently received greater attention as most of them are heat stable and amenable to proteolytic inactivation.

Lactic Acid Bacteria

LAB are a group of Gram-positive bacteria linked to a constellation of morphological, metabolic and physiological characteristics. They are included in the group of non-spore forming, non-respiring cocci or rods, catalase-negative, devoid of cytochromes; non-aerobic but aero-tolerant, fastidious, acid tolerant and strictly fermentative with lactic acid as the major end product during the fermentation of carbohydrates. LAB are widely distributed in nature. They have been isolated from grains, green plants, dairy and meat products, fermenting vegetables and mucosal surface of animals[9]; vacuum-packaged refrigerated beef[10]; sourdoughs[11] and traditional Indian fermented foods such as appam batter and vegetable pickle[12]. LAB were realized as a group of biopreservative bacteria in the beginning of the 1900's, based on their interaction in foods. The preservation factors elaborated by LAB have been identified as peptides, generally known as 'bacteriocins' apart from lactic acid, acetic acid, etc.

The increasing demand for high quality safe, processed foods has created a niche for natural food preservatives. The ideal natural food preservatives should fulfill a number of criteria such as a) acceptable low toxicity, b) stability to processing and storage, c) efficacy at low concentration, d) no deleterious effect on the food and e) economic viability. While most bacteriocins fulfill these criteria, to date nisin is the only one, commercially exploited on a large scale. Although the discovery of nisin, the first bacteriocin used on a commercial scale as a food preservative dates back to the first half of last century, but research on bacteriocins of LAB has significantly increased in the last two decades, for the search of novel bacteriocin-producing strains from dairy, meat and plant as well as traditional fermented products. Many bacteriocins have been isolated and characterized, and found to exhibit antibacterial activity against a range of pathogenic and food spoilage bacteria[10,13-16].

Bacteriocins

Bacteriocins, the anti-microbial substances of LAB have gained tremendous attention as potential bio-preservative. Bacteriocins are ribosomally synthesized, extracellularly released bioactive peptides or peptide complexes, having a bactericidal or bacteriostatic activity. In all the cases the producer cells exhibit specific immunity to the action of its own bacteriocin. Bacteriocin-producing strains can be readily identified in a deferred antagonism assay, in which colonies of the putative producer are overlaid with a bacterial lawn of a sensitive strain. After further incubation, zones of inhibition are visible in the sensitive lawn. Some bacteriocins, however, appear to inhibit potential food borne pathogens including *Clostridium botulinum, Enterococcus faecalis, Listeria monocytogenes*, *Staphylococcus aureus* and *Bacillus* species. Bacteriocins of LAB are considered to be safe biopreservatives, since they are assumed to be degraded by the proteases in gastrointestinal tract[15].

Classification

Four general classes of antimicrobial peptides or proteins (bacteriocins) from LAB have been characterized to date.

1. Lantibiotics
2. Small (<13 kDa) hydrophobic heat stable peptides

3. Large (~30 kDa) heat-labile proteins
4. Complex proteins that require additional carbohydrates or lipid 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 dehydrated serine and threonine[17]. Their distinguishing feature is the presence of post-translationally modified amino acid residues. The best example in this group 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 the more rigid ones of class Ib. Class Ib bacteriocins, which are globular in nature, have no net negative charge[18].

Class II (Small heat stable peptides)

They are bioactive peptides, which do not contain any modified amino acid residues such as lanthionine. They are also further subdivided into IIa and IIb, Class IIa includes pediocin-like bacteriocin 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. Bacteriocins composed of two different peptides comprise Class IIb, which need 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[15].

Class III (Large heat labile bacteriocins)

Heat labile proteins of large molecular weight include Helviticin-J, lactacins A and B. Meagre information is available on this group of bacteriocins.

Class IV

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 there is good reason to believe that these are artifacts due to their cationic and hydrophobic properties resulting in the formation of complexes with other macromolecules in the crude extract. This phenomenon has been demonstrated in the case of plantaricin S[15].

The majority of bacteriocins produced by the bacteria associated with food belong to Classes I and II. Among the Class I bacteriocins, nisin is the best known having GRAS status for use as a direct human food ingredient and has a broad inhibitory spectrum against Gram-positive bacteria including many pathogens and can prevent outgrowth of *Bacillus* and *Clostridium* spores. It also sensitizes spores of *Clostridium* to heat allowing a reduction in thermal process requirements. However, it is not effective against Gram-negative bacteria, yeasts and moulds. Among the Class II bacteriocins, the pediocin-like bacteriocins having anti-listerial activity are the most common. Pediocins are produced by *Pediococcus* spp. They are not very effective against spores but can inhibit *Listeria monocytogenes* effectively as compared to nisin[14,19]. Compared to other bacteriocins in the pediocin-family and to some lantibiotics, pediocin A obtained from *P. pentosaceous* FBB61 and those from *P. acidilactici* strains have a relatively wider antibacterial spectrum against gram-positive bacteria. Pediocin A inhibits the growth of several strains of *P. pentosaceous, P. acidilactici, Staphylococcus aureus, L. lactis, Lactobacillus* spp., *Cl. botulinum, Cl. perfringens* and *Cl. sporogenes*[20]. Pediocins isolated from *P. acidilactici* NCIM 2292, *P. pentosaceous* NCIM 2296 and *P. cerevisiae* NCIM 2171 are capable of inhibiting a wide spectrum of Gram-positive LAB, food borne pathogens and spoilage organisms including some of the Gram-negative organisms such as *Pseudomonas* and *E. coli*[16].

Nisin was first discovered in England in 1928 as a result of difficulties experienced during cheese making. Storage of the milk had allowed the contaminant organisms to grow and produce the inhibitor. The protein was isolated and given the name 'nisin' from its sero-group determined by the Lancefield sero-typing scheme for streptococci. Nisin is a 'Group N Inhibitory Substance'. The potential application of nisin in food preservation was first demonstrated in 1951[21] and later its uses as a food preservative were elaborated[22,23]. Aplin and Barrett have commercialized the nisin concentrate as 'Nisaplin', which is used as a preservative in milk and dairy products, canned foods, cured meats and other segments of fermentation industry.

Nisin is a polypeptide of 34 amino acid residues with a MW of 3354 Daltons and is synthesized as pre-pro-peptide of 57 amino acids, the first 27 residues of which make up an N-terminal leader sequence. The 34 residue C-terminal region contains serine, threonine and cysteine in the positions predicted, to generate the modified amino acids such as dehydroalanine, dehydrobutyrine, lanthionine and methyllanthionine present in the mature nisin. Nisin normally occurs in the more stable dimer form (7-kDa) and can also occur as a tetramer (14-kDa). It is extremely resistant to heat. It is soluble in dilute acids and stable to boiling in such solutions. It exhibits antimicrobial activity towards a wide range of Gram-positive vegetative bacteria, and is particularly effective[23].

Nisin-producing *Lactococci* occur naturally in raw milk and cheese. A worldwide survey of raw milk samples reported that 43% of them contained *Lactococci*. Inadvertently and apparently harmlessly, humans and animals have been consuming this bacteriocin for centuries. Toxicological studies have now confirmed that nisin is not toxic, even at levels much higher than those used in foods. Digestive enzymes rapidly inactivate nisin and consequently they cannot alter the bacterial micro flora in the intestinal tract. The LD_{50} value is about 7 g/kg body weight; similar to that of common salt. As the preparation tested contained 75% salt, the toxicity can be attributed to the salt component alone. No ill effects were observed in pigs and poultry from feeding experiments. There was no evidence of cross-resistance to antibiotics used in medicine. In 1969, the FAO/WHO Expert Committee permitted an unconditional acceptable daily intake (ADI) of 3.3×10^4 units/kg body weight. In 1988, the US Food and Drug Administration affirmed nisin as GRAS for use as a direct ingredient in human food[23].

The Class II bacteriocins such as pediocins are produced by the genus *Pediococcus*. Strains of *P. pentosaceous* and *P. acidilactici*, naturally found in many plant sources, have been extensively used for a long time, both in natural and pure cultures (as starter cultures) fermentations to produce fermented foods of plant, meat, fish, cereal and dairy origin[24]. Their cells are spherical, appear as tetrads and pairs, but never single cells or chains. They are Gram-positive, non-motile, facultative anaerobes and homolactic fermentative. Several types/strains of *P. pentosaceous* and *P. acidilactici* have almost identical and overlapping biochemical and physiological characteristics and thus, it is often difficult to accurately identify an isolate at the species level without performing DNA/DNA hybridization. The two species differ by only 20 to 30% in DNA homology. However, based on the characteristics of the respective 'type strains', *P. acidilactici* can arbitrarily be differentiated from *P. pentosaceous* species by its ability to grow at 50°C and survive at 70°C for 10 min, but inability to ferment maltose[20,25]. Gram-positive bacteria that produce pediocins and other bacteriocins probably have growth advantages by completely killing bacteria that are likely to be growing in the same ecological niche[26,27]. Because of this, many bacteriocins have relatively narrow bactericidal spectrums; some are effective only against a few strains of the same species of producer strain or may be a few closely related species. In contrast, pediocins of both *P. pentosaceous* FBB61 and *P. acidilactici* strains have a relatively wide spectrum that includes many food borne spoilage and pathogenic bacteria. Pediocin PA-1/AcH has been shown to produce zones of inhibition against strains of *L. monocytogenes, B. cereus, Cl. perfringens, Cl. botulinum, Cl. laramie*, and many *Lactobacillus, Leucnostoc, Enterococcus, Pediococcus* and *Lactococcus* species. It is also effective against many strains of sub-lethally stressed Gram-positive and Gram-negative spoilage and pathogenic bacteria[20]. Pediocins isolated from *P. acidilactici* NCIM 2292, *P. pentosaceous* NCIM 2296 and *P. cerevisiae* NCIM 2171 were shown to inhibit a wide spectrum of Gram-positive LAB, food borne pathogens and spoilage organisms including some of the Gram-negative organisms such as *Pseudomonas* and *E. coli*[16]. These pediocins showed significant inhibition against *B. cereus, L. monocytogenes* and *S. aureus* apart from LAB, moderate against *E. coli* and *Pseudomonas* sp. and less against *Clostridium perfringens*.

Currently, only nisin has been approved for food use. Its use is limited to acidic foods because of low solubility and stability at neutral and high *p*H values. Several bacteriocins from *Pediococcus* species have been characterized biochemically and genetically and in a number of cases their mode of action has also been studied[16,20,28]. Besides, pediocin has been shown to be more effective than nisin against some of the food borne pathogens such as *L. monocytogenes, S. aureus*, etc[29,30]. In recent years, *L. monocytogenes*, an emerging pathogen has caused severe illness from food ingestion and therefore, drawn the attention of several investigator's to focus their studies on the anti-listeria activity of bacteriocins from *Pediococcus* species[29,30] and *Lactobacilli*[8,11,12]. Since there are difficulties in using nisin for raw meat applications on account of its poor solubility and instability at the *p*H of raw meat, the use of other bacteriocins has been examined. The most promising results in meat have been obtained with pediocin PA-1/AcH from *P. acidilactici*[15]. In spite of greater interest for its use as food biopreservatives, pediocin has yet neither been legally approved by the regulatory agencies nor available commercially.

Mode of Action

Most of the bacteriocins are bactericidal, with some exceptions (leucocin A-UAL 187 being bacteriostatic). Nisin has been proposed to act on the cytoplasmic membranes of Gram-positive bacteria to cause lesions[31]. Following nisin treatment, whole or intact sensitive cells and membrane vesicles exhibit efflux of amino acids and cations. Loss of these substances depletes proton motive force, which ultimately interferes with cellular biosynthesis. These events result in collapse of the membrane potential and ultimately cause cellular death[32]. Similarly, other bacteriocins such as lactococcin A, pediocin JD, etc. have also been reported to cause dissipation of the membrane potential and increase in membrane permeability to ions leading to collapse of proton motive force[33,34]. The inhibition kinetics using the bacteriocins from *L. acidophilus* and *L. casei* indicated a bactericidal mode of action without causing cell lysis[12], similar to the observations made by Du Toit *et al*[35] with enterocins. On the other hand, the effect of the bacteriocin from *L. plantarum* NCIM 2084 was shown to be bactericidal causing cell lysis that appears to be different from the earlier characterized bacteriocins such as plantaricins C, F and KW 30[36-38], while plantaricin from *L. plantarum* LC74 showed a bacteriostatic effect[39]. To state the bactericidal or bacteriostatic mode of action of the antimicrobials, several factors such as assay systems used, concentrations and purity of the inhibitor, the sensitivity of the indicator species, the density of the cell suspension used and the type of buffer or broth used need to be considered.

Genetic Analysis

Initial genetic studies implying association of bacteriocin determinants with plasmids have been backed by physical data. This has included the cloning of genes involved in bacteriocin production and immunity, nucleotide sequence determination, and in certain cases expression of isolated gene(s).

Location of nisin gene varies among the strains of *Lactococci*. Nisin production and immunity can be mediated by plasmid DNA in *L. lactis* subsp. *lactis*. However, some results indicate that either chromosomal DNA or a conjugal transposon mediates production. Genetic determinants for nisin production and immunity have been transferred conjugally with expression of the active protein. Genes for production of and immunity to nisin are located in an operon of at least 8.5 kb. The nucleotide sequences of genes encoding the peptide precursor, pronisin, and immunity determinants have been deduced. The nisin structural gene is part of a polycistronic operon that probably includes a single promoter located upstream from the structural gene and is responsible for initiation of transcription. The primary translation product from the sequence of the nis A structural gene is a 57-amino acid residue pronisin peptide. The 34-amino acid residue C-terminal portion appears in active nisin. The remaining 23 residue N-terminal portion is removed by enzymatic cleavage at the cell surface of *L. lactis* subsp. *lactis*[32].

Cloning and nucleotide sequence determination of the plasmid-encoded pediocin PA-1 determinants identified four genes arranged in an operon-like structure. Expression of genes from this region, when cloned in a recombinant plasmid and introduced into *E. coli*, gave rise to pediocin production and secretion. These experiments also indicated that whereas ped B was apparently not required for antimicrobial activity, ped D does play a role in bacteriocin production[40].

Recombinant DNA technology is currently being applied to enhance production by transferring bacteriocin genes to other species, as well as for mutation and selection of bacteriocin variants with increased and/or broader activity spectra[41]. The development of heterologous expression systems for LAB bacteriocins may offer a number of advantages over native systems, such as facilitating the control of bacteriocin gene expression or achieving higher production levels, help to overcome problems of poor adaptability and low production or genetic instability. In addition, these heterologous systems could be used for the production of hybrid bacteriocins with improved properties[42].

Applications of Bacteriocins for Biological Preservation of Foods

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 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.

Several possible strategies for the application of bacteriocins in the preservation of foods may be considered:

- Inoculation of the food with LAB (starter or protective cultures) that produce the bacteriocin in the product (production *in situ*);
- Addition of the purified or semi-purified bacteriocin as a food preservative;
- Use of a product previously fermented with a bacteriocin-producing strain as an ingredient in food formulation.

The type of product and the intrinsic as well as extrinsic parameters existing during processing, storage and distribution will determine the particular approach of biological preservation required. Bacteriocin production *in situ* by starter cultures has a good chance of finding applications in fermented foods. In non-fermented refrigerated products, such as minimally processed meats or prepackaged vegetable salads, only those strains producing sufficient and potent amounts of bacteriocin but no other metabolic compounds, at levels 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.

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*[43] 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[44]. Both *P. pentosaceus* and *P. acidilactici* strains have been used as starter cultures in controlled and natural fermentation of meat, fish, vegetables, cereals and dairy products[24]. Use of appropriately selected psychrotrophic LAB may reduce the risk of growth of *Salmonellae* and other vegetative pathogens during sausage fermentation, and may contribute to the inhibition of *L. monocytogenes* on some perishable meat products[45]. However, the use of protective cultures cannot compensate for poor control of manufacturing processes.

Nisin is suitable for use in a wide range of foods—liquid or solid, canned or packaged, chill or warm ambient storage. Based on target organisms, its usage falls into three broad categories: i) to prevent spoilage by Gram-positive endospore formers (particularly in heat processed food), ii) to prevent spoilage by LAB and similar organisms such as *Brocothrix thermosphacta*, and iii) to kill or inhibit Gram-positive pathogens, e.g. *L. monocytogenes, B. cereus, Cl. botulinum*. Nisin 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 best time to add nisin is at the last practical stage before heat processing (if this is part of the manufacturing process). In the manufacture of processed cheese, for example, nisin is usually added to the heated cheese at the same time as the melting salts. Nisin can also be used at higher concentrations (as a processing aid) as a spray or dip for surface decontamination. The level of nisin addition depends on the type of food, severity of heat process, *p*H, storage conditions and the required shelf-life. Nisin is often used in acidic foods, but is effective in products across a wide range of *p*H values (3.5-8.0). It seems to be a very effective preservative in liquid egg, which generally has a *p*H of 7.3 to 7.8. It is used in a variety of products including pasteurized, flavoured and long-life milks, aged and processed cheeses, and canned vegetables and soups[22]. Nisin has been utilized to inhibit undesirable LAB in wine[46] and beer[47]. In India, nisin has been permitted for use in the preservation of processed cheese and tender coconut water. A process to preserve tender coconut water using nisin as a biopreservative[48] has been developed at Defence Food Research Laboratory, Mysore.

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 *p*H 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*. Many refrigerated vacuum-packaged processed food products from meat, dairy, fish and vegetable groups contain normally psychrotropic Gram-positive bacterial strains from the genera of *Leuconostoc, Lactobacillus, Carnobacterium, Brochothrix* and *Clostridium*. They are capable of multiplying at refrigerated temperature and causing spoilage of the product. By incorporating pediocin PA-1/AcH during the formulation of the raw product, spoilage problems in the final product could be reduced[26,49,50].

In several toxicological studies, pediocin PA-1/AcH was injected in mice and rabbits subcutaneously, intraperitoneally and intravenously. The animals did not show any adverse reactions[20]. Pediocin PA-1/AcH also did not kill hybridoma cells[51]. These studies suggest that pediocin will be safe when consumed with food. Also, when consumed, the proteolytic enzymes in the digestive tract will hydrolyze it leading to no unusual products because the molecule does not have any unusual amino acids. Researchers in several countries have recognized its potential as a food preservative, especially for use in certain specific foods. However, pediocin has not yet been legally approved by the regulatory agencies in the USA, Europe and other countries where the food industry is interested to use it as a food preservative. Interestingly, FDA and USDA/Food Safety Inspection Service (FSIS) research laboratories have been studying the bactericidal effectiveness of these bacteriocins. It is expected that if the results of the studies currently being conducted in many laboratories are promising, pediocin and other bacteriocins of LAB could be approved/allowed for food uses[20].

Nisin has also been used in conjunction with other preservative measures to enhance product safety or quality. In canned foods such as vegetables, soups and puddings, nisin has been applied in conjunction with heat to successfully counter heat-resistant spores of flat-sour thermophilic bacteria. Chung and Hancock[52] have clearly demonstrated the benefits of using mixtures of nisin and lysozyme against food spoilage bacteria. A combination of nisin and some lactates has been demonstrated to be more active against *L. monocytogenes* due to synergistic action[53,54]. A combinatory treatment of nisin and a listeriophage was found to be effective in controlling *L. monocytogenes,* while it was not effective in model food systems which reflect the complexity of natural systems[55]. Many of the currently used methods of processing and preservation of foods can induce sublethal injury, and in the presence of bacteriocin, the injured cells can be killed. Similarly, spores of pediocin-sensitive bacteria, although not sensitive to pediocins, following germination and outgrowth will be killed by pediocin PA-1/AcH[20]. The evaluation of antibacterial efficiency of the two bacteriocins from LAB, nisin and pediocin AcH, has revealed that they had higher antibacterial activity when applied in combination than when either one was used alone[56]. As a part of hurdle system of preservation, nisin can also be used to prevent the growth of pathogens such as *B. cereus, Cl. botulinum* and *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 filed.

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-complexing compounds. The evaluation of antibacterial efficiency of the two bacteriocins from LAB, nisin and pediocin AcH, has revealed that they were more antibacterial in combination than when they were used alone[56]. This observation has led to propose the principles of this greater antimicrobial spectrum and can be used advantageously to design efficient natural food biopreservative(s). The use of more than one LAB bacteriocin as a combination biopreservative can be advantageous over a single bacteriocin alone[56,57]. The potential value of this property in the preservation of fermented and non-fermented food products has been recognized and prompted many research groups to direct their studies to investigations on these antagonistic proteinaceous compounds. Considerable emphasis has been placed on the identification of many other antimicrobial peptides or proteins and their biochemical properties, including spectrum of activity, production conditions, purification procedures, amino acid composition, amino acid sequence, and mode of action as well as their applicability either alone or in combination so as to reduce the severity of thermal processing required. Continued study of these aspects especially structure-function relationships of bacteriocins is necessary if their potential in food preservation is to be exploited. Further research into the synergistic effects of these natural preservatives in combination with advanced technologies such as pulsed electric filed and ultra high pressure could result in replacement of chemical preservatives, or could allow less severe processing treatments, while still maintaining adequate microbiological safety and quality in foods.

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