Chapter 5

Nisin

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Abstract

Antimicrobial peptides (AMPs) are small cationic peptides which protect their hosts against bacteria, protozoa, viruses, and fungi. Bacterial AMPs are called bacteriocins, and are produced by both Gram-positive and Gram-negative bacteria. Because of their high potency and specificity, bacteriocins are considered as promising antimicrobial agents for different applications, including food preservation and infection treatment; specially the ones produced by acid lactic bacteria species (Gram-positive). Nisin is the most intensively studied and used bacteriocin, it is found commercially available and its use is regulated in over 50 countries. Therefore, special attention is given to this bacteriocin.

Keywords: nisin, *Lactococcus lactis*, antimicrobial

1. Introduction

Antimicrobial peptides (AMPs) are small cationic peptides that protect their hosts against bacteria, protozoa, viruses, and fungi [1, 2]. Some of these peptides have also demonstrated a cytotoxic activity against tumor cells and sperm [3]. These peptides are produced by several forms of life, including bacteria, insects, plants, and vertebrates, and they have been recognized as ancient evolutionary molecules that have been effectively preserved in mammals [2, 4].

These evolutionarily conserved peptides in general constitute a highly heterogeneous group of molecules, which share common features, as the small size (20–50 aa) and cationic and

amphiphilic or hydrophobic properties [4]. Since APMs have both a hydrophobic and hydrophilic side, they are soluble in aqueous environments yet also enter lipid-rich membranes [1].

AMPs demonstrate being effective against a broad range of microorganisms, including Gram-negative and Gram-positive bacteria, fungi, and viruses [1]. In higher organisms, AMPs contribute to innate immunity, serve as a first defense line against harmful microorganisms, and may be increased with inflammation and injury in humans [1, 4]. In bacteria, the production of AMPs provides a competitive advantage for the producer in certain ecological niches, being a successful strategy to decrease the numbers of competitors to obtain more nutrients [4, 5].

Bacterial AMPs are called bacteriocins and are produced by both Gram-positive and Gram-negative bacteria. However, there are some important differences between eukaryotic AMPs and bacteriocins. Bacteriocins are often very potent, acting at pico- to nanomolar concentrations, whereas micromolar concentrations are required for the activity of eukaryotic AMPs. Most bacteriocins have a very narrow target spectrum, that is, being active against only a few species/genera closely related to the producer, whereas eukaryotic AMPs are generally less specific with a broad target spectrum [4].

Bacteriocins are often confused in the literature with antibiotics. Antibiotics are secondary metabolites synthesized by enzymes and have clinical application. Bacteriocins are ribosomally synthesized and do not alter the flora of the intestinal tract since they are inactivated by digestive enzymes [5].

Table 1 shows the main differences between bacteriocins and antibiotics, based on the synthesis, mode of action, antimicrobial spectrum, toxicity, and resistance mechanism [6].

Characteristic	Bacteriocin	Antibiotic
Application	Food	Clinic
Synthesis	Ribosomal	Enzymes
Production	Primary metabolism	Secondary metabolism
Activity	Limited spectrum	Wide spectrum
Action	Cytoplasmic membrane	Several
Toxicity	Unknown	Yes
Microbial resistance	There are some strains	There are some strains

Table 1. Major characteristics that differentiate bacteriocins of antibiotics [6].

More than 99% of bacteria can produce at least one bacteriocin, most of which are not identified. These substances may be produced spontaneously or induced and the producers are immune to it [7]. Bacteriocins are classified according to the bacterial spectrum, molecular weight, chemical structure, and mode of action [8].

http://dx.doi.org/10.5772/60932

There are two important databases relative to bacterions. One is the BACTIBASE, an open-access data repository of bacteriocin, designed for the characterization of bacteriocins. BACTIBASE is developed by the Functional Proteomics and Alimentary Bio-preservation Unit at the Institute of Applied Biological Sciences Tunis (ISSBAT), Tunisia, in collaboration with Nutraceuticals and Functional Foods Institute (INAF), Laval University, Canada (http://bactibase.pfba-lab-tun.org/main.php) [9]. The BACTIBASE contains over 200 bacteriocins sequences, most of which are the products of Gram-positive bacteria, particularly lactic acid bacteria [10].

The other database is BAGEL, a web-based bacteriocin mining tool that helps to determine the presence of bacteriocins gene from a GenBank file based on a database containing information of known bacteriocins and adjacent genes involved in bacteriocin activity (http://bagel2.molgenrug.nl/) [9]. In Gram-negative bacteria, most bacteriocins have been characterized from *Escherichia coli* and other enterobacteria, and they are often referred to as microcins (small peptides, <10 kDa) or colicins (larger peptides, 25–80 kDa) [4]. The spectrum of activity manifested by bacteriocins of Gram-negative bacteria is narrower than those produced by Gram-positive bacteria [11]. Microcins show tolerance to heat and extreme pH and are divided into two subgroups [4, 5]:

a. Class I: low molecular weight (<5 kDa) and contain posttranslational modifications. Some members of this class are microcin B17, C7, and J25.

b. Class II: larger than class I (5–20 kDa) and has little or no posttranslational modifications. This subclass includes microcin E492, colicin V, and H47.

The colicins are synthesized by over half of *E. coli* strains and also by *Yersinia pestis* (pesticins), *Serratia marcescens* (marcescins), and bacteria of genus *Shigella*, *Klebsiella* (klebicins), and *Pseudomonas* (pyocins) [11].

The bacteriocins of Gram-positive bacteria are divided into four classes, according to their genetic and biochemical characteristics [6, 12, 13], being lactic acid bacteria frequently found as producers. The classes are presented as follows:

Class I (lantibiotics): constituted by thermostable low-molecular peptides (<5 kDa) with 19–38 amino acid residues, posttranslationally modified and which have highly specific amino acid in their composition, as lanthionine and β-methyl lanthionine. Class I can be divided into subclasses based on the structure and mode of action of the bacteriocin: type A—linear molecules as nisin, subtilin, and epidermin, of which nisin is best characterized; and type B—globular molecules as mersacidin and mutacin.

Class II: consists thermostable low molecular peptides (<10 kDa) with 30–60 amino acid residues and unmodified nonlanthionine. Bacteriocins of this class do not undergo posttranslational modification. Three subdivisions have been proposed for this class. Class IIa consists of pedicine-like bacteriocins that have high specificity against *Listeria monocytogenes*. This includes leucocin mesentericin A and Y105, with 37 aa, and carnobacteriocin B2, with 48 aa. Class IIb requires the combination of two different peptides for bacteriocin activity since they cannot manifest antibacterial activity separately. Members of this group are lactacin F and

lactococcin G. The bacteriocins belonging to class IIc have a covalent bond between C and N terminals, resulting in a cyclic structure, and are represented by enterocin AS48, circularin A and reutericin 6.

Class III: consists thermolabile bacteriocins of high molecular weight (>30 kDa), complex in nature of activity and protein structure. Its mechanism of action is distinct from other bacteriocins since they promote the lysis of the cell wall in sensitive cells and therefore can receive another name, bacteriolysins. They have an N-terminal domain homologous to endopeptidase and a C-terminal domain responsible for recognition by the sensitive cell. Lactacin A and B, helveticin J and V-1829, and acidophilucin A are examples of this group.

Class IV: According to Klaenhammer [14], this class consists of complex structures containing amino acids, carbohydrates, or lipids in its composition. However, this class is not recognized by other authors [6, 15] since they were not properly purified. Information related to this class is very limited.

Cotter et al. [16] proposed a new classification, wherein bacteriocins are divided into two categories: lantibiotics (class I) and nonlantibiotics (class II), while the high molecular proteins, consisting of class III, should receive a separate designation of "bacteriolisins." The authors also suggest the extinction of class IV. Because of their great biochemical diversity, the classification of bacteriocins is still under debate, and different classifications have been suggested over the years [4].

Bacteriocin is found to have bactericidal/bacteriostatic action and is affected by various factors, including dose, level of purity, indicator/pathogenic microbes physiological conditions, and environmental factors [9]. The antibacterial effect of bacteriocins generally relies on the pore formation, despite the differences between the several types of bacteriocin. There are a variety of modes of actions to attack the target bacteria, and a single bacteriocin can possess more than one mode of action [10]. The main mode of action against target cells involves the association with membrane lipids, leading to the formation of pores [4]. Pore formation results in changes of membrane permeability, with efflux of small metabolites (e.g., ions K^+, H^+, phosphate) of the susceptible cells, leading to the destruction of electrochemical gradient, leakage of cell contents, and cell death.

Because of their narrow target spectrum and high potencies, it is believed that most bacteriocins bind specific receptors on sensitive cells. A few of such bacteriocin receptors have indeed been identified, including mannose-phosphotransferase systems (man-PTS) and lipid II [4, 17]. The man-PTS is target by class IIa bacteriocins (e.g., pediocin PA-1, enterocin P, enterocin A and sakacin P) on sensitive cells of genera such as *Listeria*, *Enterococcus*, and *Lactobacillus* [4, 17].

Some lantibiotics (class I bacteriocins) use the cell wall precursor molecule lipid II as an anchoring receptor on target cells [17]. As the role of lipid II is common in all bacteria, these lantibiotics have a relatively broad inhibitory spectrum, including a number of different genera of Gram-positive bacteria [4].

While the antibacterial activity of bacteriocins is somewhat deciphered, their antiviral activity remains to be understood [18].

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Due to their protein nature, all bacteriocins are inactivated by one or more proteolytic enzymes, including pancreatic (α-chymotrypsin and trypsin) and gastric origin (pepsin) [19], being generally harmless to the human body and surrounding environment [5]. This feature is very interesting for their use in food products.

Because of their high potency and specificity, bacteriocins are considered as promising antimicrobial agents for different applications, including food preservation and infection treatment [4, 13], especially the ones produced by acid lactic bacteria species (Gram-positive) [20]. Pure or mixed cultures of bacteriocin-producer lactic acid bacteria and bacteriocin produced by them can be used as protective system against common food spoilage bacteria and pathogens [9].

Bacteriocins can be used in three ways in food: (i) inoculating the food with lactic acid bacteria strains producing bacteriocins, (ii) the addition of purified or semipurified bacteriocin, and (iii) adding a fermented ingredient with strains of bacteriocinogenic [7]. Bacteriocin produced lactic acid bacteria used as start or coculture in food production can increase flavor and shelf-life [5]. Bacteriocins can also be used for those products that cannot be sterilized by thermal treatment, and even at freshly cut vegetables, fruits, and seed sprouts, which are consumed without cooking, this may lead to various health risk due to their contamination with pathogenic bacteria [9].

To preserve and stabilize various kinds of food, including fermented dairy products, mayonnaise-type spreads, cream, cheese products, and meat or vegetable compositions, whey from nisin-producing cultures is well documented [9]. Although many other type of bacteriocin such as subtilin, cerein, thuricin, plantaricin, etc., have been isolated and characterized from different bacteriocin producing strains of bacteria yet, to date the only commercially produced bacteriocins are nisin (*Lactococcus lactis*) and pediocin (*Pediococcus acidilactici*), and others are still in a process of getting commercial status to be used as food preservatives [9]

Since nisin is the most intensively studied and used bacteriocin, we present it in more detail in the following section.

2. Nisin

Nisin is a class I bacteriocin widely exploited and applied [13, 16, 21]. Initially, nisin was sold in England in 1953, and on the following decades, it was approved for use in over 48 countries. Nisin was considered safe for use in foods in 1969 by the Joint of Food and Agriculture Organization from World Health Organization (FAO/WHO) Expert Committee on Food Additives. In 1983, nisin was added in the European list of food additives under the number E234, and in 1988, it was approved by the US Food and Drug Agency (FDA) as generally regarded as safe (GRAS) for use in pasteurized products and processed cheeses to inhibit the growth of *Clostridium botulinum*.

Produced by species of *Lactococcus lactis* subsp. *Lactis*, nisin consists of 34 amino acid residues, with molecular weight of 3.5 kDa, and isoleucine (NH_2) and lysine (COOH) as terminal amino acids (Figure 1).

Nisin is ribosomally synthesized as a 57 amino acid peptide precursor, with 23 residues in the leader region and 34 residues in the framework region. Subsequently, through enzyme postmodification translocation, the leading region is removed; the serine and the threonine from the framework region undergo dehydration, resulting in the formation of dehydroalanine (Dha) and dehydrobutirine (Dhb), respectively. The subsequent reactions of cysteine sulfhydryl side chain with Dha and Dhb result in lanthionine thioether ring structures (Ala-S-Ala) and methyl-lanthionine (Aba-S-Aba). Finally, the active nisin is secreted and released by proteolytic cleavage of the leader peptide (23 amino acids) [20, 22].

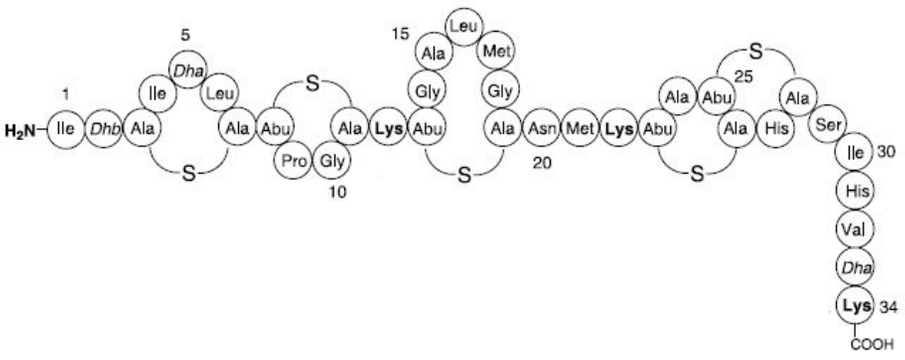

Figure 1. Schematic representation of the primary structure of nisin produced by *L. lactis* subsp. *lactis* ATCC 11454. Ala-Ala-S represents lanthionine; Abu-S-Ala-β-methyl, lanthionine, dehydroalanine Dha, and Dhb dehydrobutirine [19].

Nisin has in its composition amino acid rarely found in nature, such as dehydroalanine (Dha —02 residues), dehydrobutirine (Dhb—01 residue), lanthionine (Lan—01 residue), and methyl-lanthionine (Melan—04 residues), which can be responsible for important functional properties, as thermostability and bactericidal action [23]. Its cationic character is due to a combination of three lysine residues and one or more histidine residues [24]. Nisin is also an amphipathic molecule due to the presence of hydrophobic residues at the N-terminal region and the C-terminal hydrophilic region [19].

A natural nisin variant produced by *L. lactis* subsp. *lactis* ATCC 11454 is called nisin A and differs from nisin Z, produced by other species of *L. lactis*, by the change of the amino acid residue position 27, histidine in the nisin A to asparagines in nisin Z [25]. This substitution does not affect the antimicrobial activity but results in better properties of diffusion in the nisin Z [26]. The solubility of nisin Z is better than nisin A at a pH above 6 since the asparagine side chain is more polar than the one from histidine [27].

Nisin solubility, stability, and biological activity are highly dependent on pH, temperature, and nature of the substrate. The solubility and stability of nisin increase with increasing acidity. Thus, under neutral or alkaline conditions, nisin is almost insoluble. It is stable at thermal

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treatment and can be autoclaved at 121°C for 15 minutes, at pH 2–3 without denaturation and with an activity loss below 10% [28].

2.1. Mechanism of action, action spectrum, and toxicity effects

Nisin has antimicrobial effect against a broad spectrum of Gram-positive bacteria and spore germination but shows little or no activity on Gram-negative bacteria, fungi, or viruses. Both vegetative cells and spores are sensitive to nisin, but the spores are usually more sensitive than the vegetative form. Depending on its concentration, nisin can be bactericidal or bacteriostatic [29]

Nisin has demonstrated antibacterial activity against pathogenic bacteria such as *Streptococcus mutans, Streptococcus sanguinis, Lactobacillus acidophilus,* and *Enterococcus faecalis* and is highly active against *Listeria monocytogenes, Staphylococcus aureus, Bacillus cereus, Lactobacillus plantarum, Micrococcus luteus*, and *Micrococcus flavus* [21, 30, 31].

Nisin acts by a dual mode of action, combining both the mechanisms: pore formation, and in the vegetative cells, it interferes in the cell wall synthesis. Since it is positively charged with hydrophobic parts, electrostatic interactions with the phosphate group negatively charged from the cell membrane occur, leading to nisin connection with the target cell. Here, lipid II serves as a docking molecule and mediates a "targeted" pore formation [32]. The formation of pores in the membrane with 2–2.5 nm in diameter allows small and essential molecules (K^+, ATP, and amino acids) to leak from the cell, resulting in the disruption of the barrier function and, consequently, in the dissipation of the membrane potential. Finally, this results in the abrupt arrest of all cellular processes and in cell death [32] (Figure 2). The mechanism of forming pores is the same as the one used by other lantibiotics, as lacticin 3147, subtilin, and epidermin, among others.

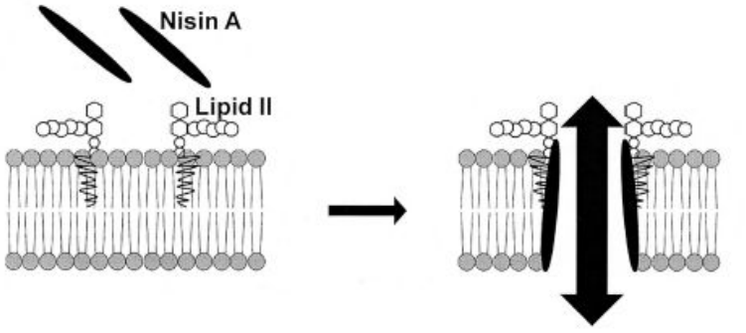

Figure 2. Nisin mode of action on a cell target. The nisin N-terminus binds to the lipid II, permeabilizes the membrane, and leads to pore formation [10].

Studies have shown that nisin also interferes with cell wall biosynthesis, by its ability to bind to lipid II, a peptidoglycan precursor, inhibiting then the cell wall biosynthesis [6, 33–36]. The N-terminal rings (A and B) form a binding pocket, the pyrophosphate cage that allows binding to the pyrophosphate moiety of lipid I/II. Complex formation with lipid II prevents transgly-

cosylation by steric hindrance and results in the sequestration of the precursors and, hence, in its abduction from the sites of nascent cell wall biosynthesis[32].

In Gram-negative bacteria, the outer layer composed of lipopolysaccharide (LPS) acts as a barrier to the nisin action on the cytoplasmic wall. However, the addition of chelating agents, such as EDTA, confines the Mg^{2+} and Ca^{+} from LPS and destabilizes the LPS layer. Therefore, nisin can be transported through the LPS layer and create pores in the cytoplasmic membrane, causing the loss of the proton motive force and intracellular nutrients [37].

The mechanism by which nisin prevents the germination of spores is different from the one that occurs in the vegetative cells. It is believed that the dehydroalanine residue (Dha) in the 5-position interacts with vital sulfhydryl groups present on the membrane of freshly germinated spores and exert a profound bacteriostatic effect, resulting in subsequent inhibition of spore. Thus, nisin allows the germination of spores but inhibits subsequent steps of the new cell formation process [38, 39]

Toxicology studies have shown that nisin intake does not cause toxic effects to the human body, and LD50 reported was 6950 mg/kg, similar to table salt, when administered orally [40]. Research conducted in the oral microflora showed that 1 minute after consuming chocolate containing nisin, only 1/40 of the original nisin activity was detected in saliva [6]. Nisin could cause hemolysis at concentrations that were 1000-fold higher than those required for antimicrobial activity [41].

Based on the "no effect" level observed in toxicological evaluations of animals and allowed for humans, the WHO recommends as the Acceptable Daily Intake (ADI, maximum amount of additive that could be ingested daily without causing any damage to consumer health) of 33,000 international units (IU) (0.825 mg) per kilogram of body weight [21].

Despite the wide application of nisin as natural food preservative, especially in dairy products, there is no agreement on the maximum levels allowed between countries where its use was legally approved, e.g., nisin can be added to cheese without limit in the UK [42].

2.2. Production and purification

Nisin production is affected by many factors such as the producer species, composition of the culture medium, pH, temperature, agitation, aeration, adsorption of nisin by the producing cells, and enzymatic degradation [43].

The release of nisin from the cells to the medium is pH dependent. At pH lower than 6, more than 80% of the produced nisin is extracellularly released. At pH higher than 6, most of nisin produced is associated to the cell membrane or intracellularly [44]. Nisin can also be reversibly adsorbed by some proteins or by the producer cell. In general, between 93% and 100% of bacteriocins are adsorbed at pH near 6.0, and the lower adsorption (≤ 5%) is around pH 1.5 to 2.0. They are also highly sensitive to the action of proteolytic enzymes [6, 45].

At laboratory scale, commercial culture media is used, such as MRS and M17 broth, but their high cost makes them impractical for large-scale production. In addition, the culture medium usually contains excess of protein (tryptone, peptone, meat extract, and yeast extract), leading to the nonconsumption of a substantial proportion and unnecessary costs and difficulties to

http://dx.doi.org/10.5772/60932

the nisin purification processes [46]. The components cost from the culture medium may vary between 38% and 73% of the total production cost, and the carbon source is the most expensive item from the medium [47].

In this sense, industrial wastes have aroused the interest to be used as raw material for bacteriocins production. Whey, a milk byproduct, discarded by the dairy industry, has been used in some researches for the production of nisin [48, 49].

Mondragón-Parada and coworkers [50] supplemented whey with minerals and small amounts of yeast extract in order to obtain lactic acid bacteria biomass production. Liu and collaborators [51] also studied a whey supplemented medium to obtain simultaneous nisin and lactic acid. Lactic acid is used in the food industry and in the production of poly lactic acid, a biodegradable biopolymer. However, the small amount of lactic acid obtained by these authors became the separation process expensive.

Commercial media, different combinations of commercials substrates at low concentrations, milk, and milk whey have been tested at microaerophilic conditions for the production of enterocin EJ97 bacteriocin. The highest bacteriocin activity was obtained using pasteurized buffered milk whey as growth substrate [52].

Penna and coworkers [53] conducted studies where shaker cultures of *L. lactis* with media containing 25% milk and 25% MRS or M17 broth were satisfactory for producing nisin.

Bioengineering techniques to improve bacteriocins production have been studied by some authors [54–57]. They report a greater production of nisin by genetically modified strains. Moreover, these techniques could improve antimicrobial activity of nisin or its stability at elevated temperature and/or under neutral or alkaline conditions.

The semipurified nisin preparations are processed by food-grade techniques. An example is Nisaplin™, a commercial preparation obtained from the fermentation of *L. lactis* on milk medium. The fermented resultant is subsequently concentrated, separated, processed by spray dryer technique, and turned into small particles. The final product consists of 2.5% of nisin contained in NaCl and denatured milk solids [58]. The amount of 1 g of this product is standardized with an activity of 10^6 IU.

Specific purification protocols were designed for nisin purification depending on its final usage (e.g., food, drug, cosmetics). Nisin has been purified using expanded bed ion exchange chromatography [59], immune-affinity chromatography [60, 61], ion exchange chromatography, hydrophobic interaction chromatography, gel filtration, and reversed-phase high-pressure liquid chromatography [62, 63]. There are also some nisin extraction protocols, using organic solvents, such as ethanol and methanol [64], or ammonium sulfate precipitation and acid precipitation (pH 2.0) [65].

Nisin is highly active in acid pH but lose activity above pH 7. It is not effective for meat preservation due to the presence of components, such as phospholipids, which limit nisin activity. High-fat content also can affect uniform distribution of nisin in food [58]. A moderate concentration of NaCl, present in many foods, is responsible for neutralizing nisin action [66]. Food additives such as sodium metabisulfite (an antioxidant) and titanium dioxide (a colorant) are frequently used in food and also affect the antimicrobial activity of nisin [67]. Nisin activity efficiency could also be affect by the contamination level of the food [20].

2.3. Nisin application

Nisin is applied as natural preservative in food, such as cheese, butter, canned, alcoholic drinks, sausages, pasteurized liquid egg, and salad dressings, among others [67], alone or in combination with other conservation methods. Other applications in preservation technologies include the development of antimicrobial active packaging [68], liposomes [69], and nanodelivery systems [70].

Although the main application of nisin is in foods, particularly dairy products, research have found its potential for therapeutic purposes, such as treatment of atopic dermatitis [71], stomach ulcers, and colon intestinal infections for patients with immunodeficiency [72, 73]. Researchers have shown the effectiveness of the antimicrobial activity of nisin in control of respiratory tract infections caused by *S. aureus* in an animal model [74]. Fernández and coworkers [75] studied the use of nisin as an efficient alternative to antibiotics for the treatment of staphylococcal mastitis in women during lactation.

Aranha et al. [76] studied the nisin application as vaginal contraceptive for humans. Gupta et al. [77] continued the research and showed no toxicity evidence for nisin has, suggesting its potential clinical application as a prophylactic vaginal contraceptive for women. Nisin has also been evaluated for use in toothpaste [78]. Others examples of nisin application are describe in Table 2.

Area	Application	Studies
Endodontic	Dental caries treatment and root canal infection	[31]
Food control	Oil emulsions, cheese preservative	[79, 80]
Nanotechnology	Coated antibiolfim, food biopreservative	[70, 81]
Pharmaceutical industry	Herpes treatment, respiratory tract infection	[3, 74]
Veterinary	Mastitis	[82]

Table 2. Examples of nisin applications.

Nisin represents an advance for the microbiological safety in dairy products. Further, research is needed to identify drawbacks that may affect their future applications, as the development of innovative in the pharmaceutical, nanotechnological, and medical areas.

3. Conclusion

Increased interest has been shown in antimicrobial peptides (AMPs), especially as an alternative to antibiotics. However, because many AMPs are toxic to mammalian cells, they are not good candidates for therapies [83]. Bacteriocins, AMPs produced by bacteria, have shown little

or no toxicity to humans, especially the ones produced by lactic acid bacteria (LAB). Bacteriocins possess vast potential as additive or as substitute for existing antimicrobial compounds, which can be used in formulation of different food products [9].

The most studied and interest bacteriocin from LAB is nisin, which is currently used in over 50 countries as a food preservative. Nisin holds many characteristics and chemical properties, like low molecular weight, thermal stability, lack of toxicity, low tendency to generate resistance, and low immunogenicity, which make it suitable for potential applications in different health care associated settings such as in human and veterinary medicine and in biochemical, pharmaceutical, agricultural, or food industries [32]. Nisin has been employed as a biological food preservative in processed dairy products, canned fruits, and vegetables for more than 50 years [32]. The potential of bacteriocins as promising alternatives for traditional antimicrobial therapeutics, as probiotics or preservatives in different areas of the healthcare sector and in associated industries, has been discussed for many years. So far, only nisin has been successfully introduced as a preservative into food industry or as a prophylactic in veterinary settings [32]. Moreover, nisin has a potential role for a new product that is cost-competitive and also provides high quality for them, especially for products having strict specifications of safety and quality supervision.

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