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Chapter

Bacteriocins: Applications in Food Preservation and Therapeutics

Parul Thapar and Mohinder Kumar Salooja

Abstract

The awareness in preventing the use of chemical preservatives for food has increased. Not only this, but the prevalence of antimicrobial resistance in the food-borne pathogens that can cause infections such as food poisoning is also at a rise. This has led in the growing demand for the safe food. The bacteriocins can be used as an effective alternative in food preservation and safety. Bacteriocins are ribosomally synthesized proteins that possess certain inhibitory activities against diverse group of undesirable microorganisms. These are produced by both Gram-positive and Gram-negative bacteria and some of the archaeal species. Bacteriocins are safe for human consumption, since they can be degraded by proteolytic enzymes in the gastrointestinal tract. In this chapter, focus is made on an alternative and safe approach for food preservation and therapeutics through bacteriocins. The applications of different types of bacteriocins in preserving food are mentioned with regard to increased shelf life, additives, and packaging. Not only this, but also bacteriocins benefit in boosting the immune system and possess certain anticancer properties. Bacteriocins can also be used in controlling the antimicrobial resistance in certain food-borne pathogens. They are the future antimicrobial proteins for the food preservation and therapeutics in a cost-effective manner.

Keywords: bacteriocins, shelf life, food preservation, immune system, *Lactobacillus* spp., therapeutics, antimicrobial resistance

1. Introduction

According to the present scenario, where people have become health and diet conscious, the use of chemical preservatives in foods has become a concern in a healthy lifestyle [1]. Another concern is the antimicrobial resistance of the food-borne pathogens within the food that can lead to spread of infections such as food poisoning [2].

Antimicrobial resistance is the ability of the microorganisms (bacteria, protozoa, fungi, or virus) to continue to grow even when they are exposed to antimicrobial medicines that are meant to kill or inhibit their pathogenic activities. As a result, medicines become ineffective and a person is not cured. It mainly happens due to overuse of the same medicine against a specific disease, which let the genes of the microbes to get adapted to a particular medicine [2]. Antimicrobial resistance is increased in various microorganisms that can lead to food spoilage and cause severe

infections. The antimicrobial resistance of these food-borne pathogens in food-producing animals can be spread to humans *via* contaminated food or water and also through direct contact with the animals [3]. This focuses on “One- Health Concept,” which is an approach that recognizes “the health of people is closely connected to the health of an animal” [4].

In one of the studies conducted by European Union, it is shown that the *Salmonella* spp. isolated from turkeys, meat, and pork showed antimicrobial resistance to the drugs such as sulfonamides, tetracycline, ampicillin, and fluoroquinolones. Also, the species of *Escherichia coli* isolated from meat and turkey showed antimicrobial resistance to sulfonamides, tetracycline, and ampicillin drugs against the patients suffering from food poisoning after consumption of these animal foods [5].

According to the Centre for Disease Control and Prevention (CDC), the antimicrobial resistance of pathogens from the family Enterobacteriaceae, including *Escherichia coli*, *Shigella*, and *Salmonella* spp., poses a serious threat to the world [4].

The increase in the demand of natural preservatives to be used in food products and natural sources that can inhibit antimicrobial resistance has led the researchers to think about different approaches toward food preservation and safety. Therefore, the application of bacteriocins can be an effective alternative.

Bacteriocins are ribosomally synthesized antimicrobial proteins [6]. These are produced by both Gram-positive and Gram-negative bacteria and some of the archaeal species. They possess certain inhibitory activities against diverse group of undesirable microorganisms [7].

The bacteriocins from Gram-positive bacteria show the following characteristics—antimicrobial in action, narrow spectrum, active against relative species of organisms, and in broad spectrum, active against both Gram-positive and Gram-negative organisms and some fungi [8]. The large group of microbial species producing bacteriocins is mainly the lactic acid bacteria (LAB). These are a group of Gram-positive, non-spore forming, non-motile, non-respiring bacteria, which produce a variety of antimicrobial compounds such as lactic acid, acetic acid, ethanol, formic acid, fatty acids, hydrogen peroxide, and bacteriocins [9]. The genera includes *Lactobacillus*, *Leuconostoc*, *Pediococcus*, *Streptococcus*, *Alloiococcus*, *Carnobacterium*, *Dolosigranulum*, *Enterococcus*, *Oenococcus*, *Tetragenococcus*, *Vagococcus*, *Weissella* [10].

Bacteriocins can be considered as an alternative in food preservation compared to chemical preservatives. These are safe for human consumption, since they can be degraded by proteolytic enzymes in the gastrointestinal tract [6]. Bacteriocins become inactive when they come in contact with the digestive enzymes in the stomach, as the enzymes such as pepsin denature the bacteriocins [9]. Also, presently, 50 LAB strains have obtained the Qualified Presumption of Safety (QPS) status by the European Food Safety Agency [11].

2. Classification of bacteriocins

Different types of bacteriocins have been classified according to size, inhibitory mechanism, target cells, spectrum of action, interaction with immune system, and biochemical features [12]. The bacteriocins have different mechanisms of action: bactericidal, with or without cell lysis through cell wall, and bacteriostatic, inhibiting the cell growth by inhibiting gene expression or protein production [6]. Accordingly, the types of bacteriocins are represented in **Table 1**.

Class	Typical producing species	Properties	Examples	Mode of action	References
I	<i>Lactococcus lactis</i> sub sp. <i>Lactis</i>	Contain unique amino acids, that is, lanthionine and methyllanthionine; <5 kDa	Nisin, Lactocin, Mersacidin	Bactericidal; by targeting the peptidoglycan layer of the bacterial cell wall. Except nisin—targets by both mechanisms	[12–14]
			Some novel lanthionines—linardin, azoline, cyanobactin, glycocin, , thiopeptide, and lasso peptide	Bactericidal	[15]
IIa	<i>Leuconostoc gelidum</i>	Heat stable, non-modified, cationic, hydrophobic peptides; contain a double-glycine leader peptide; pediocin-like peptides; <10 kDa	Pediocin PA1, Sakacin A, Leucocin A		[13, 16, 17]
IIb	<i>Enterococcus faecium</i>	Require synergy of two complementary peptides; mostly cationic peptides	Lactococcin G, Plantaricin A, Enterocin X		[18, 19]
IIc	<i>Lactobacillus acidophilus</i>	Affects membrane permeability and cell wall formation	Acidocin B, Enterocin P, Reuterin 6		[20, 21]
III	<i>Lactobacillus helveticus</i> from Swiss cheese	Heat-labile; large molecular mass peptides; >30 kDa	Lysostaphin, Enterolysin A, Helveticin J	Bacteriostatic	[13–15, 19, 21]

Table 1.
 Classification of bacteriocins.

3. Applications of bacteriocins in food preservation

Bacteriocins have been used in biopreservation of various foods, either alone or in combination with other methods of preservation, such as hurdle technology [19]. The criteria for the bacteriocins to be used for food preservation or food safety are [22, 23]:

- Bacteriocin-producing strains should be food grade (GRAS or QPS).
- Exhibit a broad spectrum of inhibition.

- Show high specific activity.
- Have no health risks.
- Beneficial effects on foods (e.g., improve safety) and do not affect quality and flavor of food.
- They should be heat and pH stable.

Bacteriocins can be incorporated in foods either by inoculating directly into the food or incorporating in food packaging films/coatings, which will improve their activity or stability in complex food systems [24].

To protect the foods from contamination with certain microorganisms such as *Listeria monocytogenes* (pathogens in cheese) and *Streptococcus aureus* in dairy products [25] while in meat and fermented sausages, contamination of *Clostridium botulinum* [26–28] and other bacterial pathogens such as *Campylobacter* spp.,

S.No.	Bacteriocin-producing strains	Products incorporated	Active against	References
1.	<i>Lactococcus lactis</i> 8L1A and 8L1 B	Starters for cheese	Bacterial pathogens	[29–31]
2.	<i>Lactobacillus sakei</i> sub sp. <i>sakei</i> 2a	Cheese spread	<i>Listeria monocytogenes</i>	[21, 32]
3.	<i>Lactiplantibacillus plantarum</i> CCDM1078	Cheese spread	<i>Listeria monocytogenes</i>	
4.	<i>Staphylococcus equorum</i> SE3	Cheese	<i>Listeria monocytogenes</i>	[33]
5.	<i>Enterococcus faecium</i>	Fresh cheese whey	<i>Listeria monocytogenes</i>	[34]
6.	<i>Lactococcus lactis</i> (Nisin producer)	Fresh cheese	<i>Listeria monocytogenes</i>	[35]
7.	Enterocin from <i>Enterococcus faecalis</i> LBB1K3	Model fresh cheese	<i>Listeria monocytogenes</i>	[36]
8.	Lacticin 481 (<i>Lactococcus lactis</i>)	Fresh cheese	<i>Listeria monocytogenes</i>	
9.	Semi -purified pediocin	Fermented cheese	<i>Staphylococcus aureus</i>	[37]
10.	Semi-purified lacticin	Fresh cheese	<i>Listeria monocytogenes</i>	
11.	Semi-purified bacteriocin—BacFL31	Turkey meat	<i>Listeria monocytogenes</i> and <i>Salmonella typhi</i>	
12.	<i>Enterococcus faecium</i> KEB2	Raw and sterile milk	<i>Listeria monocytogenes</i>	
13.	Aureocin A70 (<i>Staphylococcus aureus</i>)	UHT skim milk	<i>Listeria monocytogenes</i>	[38]
14.	Lactococcin BZ (<i>Lactococcus lactis</i>)	Skim and UHT milk	<i>Listeria monocytogenes</i>	[39]
15.	<i>Leuconostoc mesenteroides</i> , Leucocin K7	UHT and whole-fat milk	<i>Listeria monocytogenes</i>	[40–42]

Table 2.
Bacteriocins-producing strains used as biopreservatives.

S. No.	Bacteriocin-producing strains	Food products	Active against	References
1.	Nisin	Cured meat products	<i>Listeria monocytogenes</i> , <i>Clostridium spp.</i> , <i>Bacillus cereus</i> , <i>Escherichia coli</i> , and <i>Salmonella spp.</i>	[43]
2.	Plantaricin 423	Cheese	<i>Listeria innocua</i>	[44]
3.	Nisin A, nisin Z and lacticin-481	Cottage cheese	<i>Listeria monocytogenes</i>	[45, 46]
4.	Nisin A (Nisapsin ^R)	Milk pudding	Spore formers	[47]
5.	Nisin A (Nisapsin ^R)	Cheese	<i>Staphylococcus aureus</i>	[48]
6.	<i>Lactococcus lactis</i> N564, Nisin	Cow milk	<i>Listeria monocytogenes</i> and <i>Staphylococcus aureus</i>	[49]

Table 3.
Some commercialized bacteriocins.

Salmonella spp., *Escherichia coli*, *Listeria monocytogenes*, *Staphylococcus aureus*, *Yersinia enterocolitica* [29]; some viral and parasitic species such as *Toxoplasma gondii* and *Taenia solium* [30], certain immobilized form of bacteriocins have been developed as antimicrobial packaging films [26–28].

3.1 Bacteriocins as biopreservatives

Some of the bacteriocin-producing strains are used as biopreservatives in food preservation but not marketed yet. These are shown in **Table 2**. There are other bacteriocins that have been proposed for industrial applications such as Enterocin AS-48 [26] and Lacticin 347 [41].

Till present, there are some bacteriocins that have been commercialized as food additives including nisin, with trade name Nisaplin and Danisco; pediocin A1, with trade name MicrogardTM and ALTA2431 [42]. The other commercialized bacteriocins active against certain food spoilage microorganisms are represented in **Table 3**.

3.2 Bacteriocins as food additives

Food additives are the substances added to food to maintain or improve the safety, freshness, taste, texture, or appearance. Food additives need to be checked for potential harmful effects on human health before they can be marketed [50]. Some of the bacteriocins that are applied as food additives are presented in **Table 4**.

S.No.	Bacteriocin-producing strains	Food additives	References
1	Bacteriocin-producer adjunct of <i>Pediococcus acidilacti</i>	Cheddar and semi-hard cheeses	[44]
2.	Bacteriocin-producing adjunct of <i>Lactobacillus acidophilus</i>	Food fermentation	[49]

Table 4.
Bacteriocins as food additives.

Bacteriocin-producing strains	Food packaging	Features	References
Nisin coated on polyethylene films	Packaging of poultry	Reduced risk of <i>Salmonella</i> spp.	[51]
Nisin absorbed onto salinized silica surfaces	Packaging of meat	Inhibited <i>Listeria monocytogenes</i>	
<i>Lactocaseibacillus casei</i> producing bacteriocin strain	The bacteriocins adsorbed on the packages on cheese, diffused through the medium, thus, inhibiting the growth of undesirable organisms like <i>Escherichia coli</i> and <i>Staphylococcus aureus</i>	Reduced the risk of pathogen development and extending the shelf-life of foods.	[21, 52]

Table 5.
Bacteriocins in food packaging.

3.3 Bacteriocins in food packaging

Antimicrobial packaging film prevents microbial growth on food surface by direct contact of the package with food surfaces such as meat and cheese [51]. In one of the studies, bacteriocin-producing lactic acid bacteria were isolated from Yakult (a probiotic drink) to develop an antimicrobial packaging (Table 5) [52].

4. Applications of bacteriocins as therapeutics

4.1 Bacteriocins in boosting immune system

Bacteriocins play an important role in boosting the immune system of the human beings. They allow the survival of specific bacterial strains in the

S. No.	Bacteriocins	Immune system, disease prevention, and Anticancer properties	Reference
1.	Bactofencin A or bacteriocin 21 produced by <i>Enterococcus faecalis</i>	Kill multidrug-resistant bacteria	[53]
2.	Pyocin	Control <i>Pseudomonas</i> lung infections in patients with cystic fibrosis.	
3.	Colicin A and Colicin E1	Inhibitory activity against the growth of eleven different tumor cell lines	
4.	Colicin D and Colicin E2	Inhibitory effect against murine leukemia cells P388	
5.	Colicin E3	Suppressed the malignant transformation of a chicken monoblast line	
6.	Bacteriocins of <i>Escherichia coli</i>	Act against human colorectal carcinoma cells	
7.	Nisin (commercial)	Act against human colorectal carcinoma cells	
8.	Bacteriocins from lactic acid bacteria	Inhibits vancomycin-resistant <i>Enterococci</i> , <i>Salmonella enteritidis</i> , <i>Clostridium difficile</i> , and <i>Listeria monocytogenes</i>	[54]

Table 6.
Bacteriocins in immune system.

S.No.	Bacteriocins or producer strains	AMR-resistant strains	Reference
1.	Nisin	Methicillin-resistant <i>Staphylococcus aureus</i> (MRSA) in goat milk	[55]
2.	Lip A	<i>Pseudomonas aeruginosa</i>	[56, 57]
3	BAC-1B-17 from <i>Bacillus subtilis</i> (thermostable) and Sonorensin from <i>Bacillus sonorensis</i>	Methicillin-resistant <i>Staphylococcus aureus</i> (MRSA)	[58, 59]
4.	Strains of <i>Lactobacillus helveticus</i> , <i>Lactobacillus delbreuckii</i> , <i>Lactococcus lactis</i>	Vancomycin-resistant <i>Salmonella enteritidis</i> , <i>Clostridium difficile</i> , <i>Listeria monocytogenes</i>	[60, 61]

Table 7.
Bacteriocins in inhibiting antimicrobial-resistant (AMR) food pathogens.

gastrointestinal tract. For example, some strains of *Lactobacillus* spp. are able to resist modification by the host diet; also inhibit other killing factors and colonization by pathogenic species within the intestine. Hence, they improve the gut barrier function and host immune response. Bacteriocins also possess anticancer properties by inhibiting the growth of the tumor cells in some animals and humans. The examples of bacteriocins in immune system and anticancer properties are mentioned in **Table 6**.

4.2 Bacteriocins in inhibiting antimicrobial-resistant (AMR) food pathogens

The bacteriocins inhibiting the antimicrobial-resistant strains of the microbes present within the food work on the principle of quorum sensing. They inhibit the competitive strains by directly influencing the niche competition [55]. The bacteriocins or producing strains that can inhibit the antimicrobial-resistant food pathogens are mentioned in **Table 7**.

5. Conclusion and future scope

Bacteriocins are the antimicrobial proteins, which could be categorized as antibiotics, but they are not. The major difference between bacteriocins and antibiotics is that the bacteriocins are species-specific and their activity is restricted to a particular strain of species; on the other hand, antibiotics have a wider activity spectrum. The bacteriocins are produced by the lactic acid bacterial species. The increased antimicrobial resistance and growing awareness of microbiome for the importance of human health underscore the need of this class of antimicrobials, as an approach for the treatment of infectious diseases spread by the antimicrobial-resistant food-borne pathogens such as *Salmonella* spp., *Listeria* spp. etc. [62]. Thus, these bacteriocins can be a part of sustainable development goals by delivering safe foods with longer shelf life [1]. The bacteriocins are the future antimicrobial proteins for the preservation of food and as therapeutics even in a cost-effective manner. The species of Enterococci have been identified as antimicrobials against vancomycin-resistant pathogens of the food industry [63, 64]. The food and dairy industries and healthcare sector should be more focused on the use of bacteriocins for food preservation and as therapeutics in cancer treatment, respectively.

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