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Research Progress and Potential Applications of Bacteriocins in the Food Industry

Zhenghao Wang[1, *]

[1] College of Food Science and Engineering, Northwest A&F University, Yangling, Shaanxi Province 712100, China

[*] Corresponding author: wang.z.h317@nwafu.edu.cn

Abstract:

Bacteriocins represent a class of secondary metabolites consisting of short peptides with potent bacteriostatic activity and are derived from both Gram-positive and Gram-negative bacterial species. Currently, they are under intense scrutiny in the scientific community. Historically, limited research on bacteriocins, coupled with insufficient understanding of their toxicity signatures and mode of action, has hampered the exploration of antimicrobial resistance. However, recent breakthrough discoveries and validations related to the taxonomic, biosynthetic, and mechanistic foundations of bacteriocins have significantly advanced the development of this field, laying a solid foundation for their development in food preservation, medical, and biological applications. In this comprehensive review, the aim is to present the current status of bacteriocin sources, their different classification schemes, and the complex mechanisms that underpin their biological activity. In addition, this review provides promising avenues for the future application of bacteriocins in the food industry, the medical sector, and the biological sciences, highlighting their potential to revolutionize these fields.

Keywords: Bacteriocins; lactic acid bacteria; lanolin antibiotics; colicin.

1. Introduction

Foodborne pathogens are an important cause of many diseases, in addition, because many foods will grow some mold and rot microorganisms, which will bring huge economic losses and damage to human health. In addition, in the food industry, antibiotics and chemical preservatives are used indiscriminately and inappropriately to inhibit or kill these mold and rot microorganisms, but excessive use can lead to the emergence and development of drug-resistant bacteria, which makes these problems more serious [1]. As a result, the food and more industries are constantly in need of new natural and safe antimicrobial drugs to combat these problems. Bacteriocin has attracted a lot of attention in recent years.

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Bacteriocins are a class of polypeptides or proteins with bacteriostatic activity produced by certain bacteria through ribosome synthesis mechanisms during metabolism. For the same closely related strains exhibiting a narrow antibacterial spectrum, bacteriocins directly degrade the DNA of target cells by perforating on target cells, inhibiting peptidoglycan synthesis, and binding to ribosomes or tRNA to inhibit protein synthesis, thereby exerting bacteriostatic effects. In many studies, bacteriocins have a narrow antimicrobial spectrum that can only inhibit or kill bacteria that are genetically closely related, which is not conducive to the study of preservatives used in food. For example, Nisin is allowed to be added to foods in more than 50 countries as a biological preservative, but some studies have found that Nisin does not inhibit the growth of certain pathogenic microorganisms in some foods [2]. However, in some studies, researchers have found and verified that the bacteriocins produced by some microorganisms have a broad-spectrum antibacterial effect, and have an inhibitory effect on multidrug-resistant strains.

For example, some researchers have discovered the presence of lactic acid bacteria (LAB) in the traditional cuisine of Xixian County, Shaanxi Province, China, which can not only help protect food and improve food safety, but also help to produce the desired flavor [3]. Other researchers have discovered that there is also a LAB in sour camel milk fermented in Xinjiang, and the ability of this LAB to preserve is due to the production of antimicrobial metabolites such as bacteriocins, diacetyl, hydrogen peroxide and organic acids [4]. There are a variety of strains in LAB that can produce bacteriocins, and after these bacteriocins have been purified, characterized, and sequenced, these bacteriocins have been found to have a broad antimicrobial spectrum and have great potential to be food biopreservatives.

This article will introduce several bacteriocins with a broad antimicrobial spectrum, and analyze the current problems of these bacteriocins if they are to replace chemical preservatives, as well as their future use in the food industry.

2. Synthesis of Bacteriocins

2.1 Source of Bacteriocins

Fermentation has been passed down and changed over the years and is everywhere around us. People use fermentation to apply to food, fermentation is to use the natural microorganisms in food to extend the storage time of food, change the nutritional value of food and improve the flavor of food. Fermented foods are rich in probiotics, among which LAB is the most common, in addition to their potential application as probiotics, they can also produce antimicrobial peptides to inhibit the growth of microorganisms.

2.2 Classification of Bacteriocins

The bacteriocin, commonly produced by E. coli, is a typical gram-negative bacteriocin; The bacteriocins produced by LAB, known as lactic acid streptococcins, are gram-positive bacteriocins. There are many types of bacteriocin produced by gram-positive bacteria, which can generally be divided into four categories [5].

2.2.1 Gram-Positive Bacteriocins

The bacteriocins that have been discovered so far can be divided into four categories according to their chemical structure, stability and molecular weight. The first category is defined as lanolin antibiotics, which are a class of small molecule modified peptides, containing more than 19-50 amino acids, with good thermal stability, and the molecular weight is generally less than 5kDa. The second class is also small-molecule, heat-stable peptides, typically less than 10 kDa, and is hydrophobic and membrane-active [5]. The second group of bacteriocins can be subdivided into three subclasses, of which the first N-terminal amino acid sequence is Tyr-Gly-Asn-Gly-Val, and consists of two cysteines to form an S-S bridge, which is active against Listeria (mainly found in fresh foods and is a foodborne pathogen) [6]. The second is that the pore complex is formed by two peptide oligomers with different amino acid sequences. The third can be activated by thiols, and the active group requires an essential cysteine residue[5]. The third type is heat-sensitive macromolecular proteins, the molecular mass is generally greater than 10kDa, and it will be inactivated after 30s at a temperature of 100 °C or lower, but this class of bacteriocins has a narrower antibacterial spectrum. A fourth type of complex macromolecular complex, which contains carbohydrates or lipid groups in addition to proteins, has not yet been purified [5]. Class 2, 3 and 4 bacteriocins generally do not contain lanolyphur amino acids, so they are also commonly referred to as non-lanolin sulfur antibiotics.

2.2.2 Gram-Negative Bacteriocins

At this stage of research so far, there is little information about bacteriocins produced by gram-negative microorganisms, and the classification is limited to two. In general, gram-negative bacteriocens are mainly isolated from Escherichia coli or other enterobacteriaceae. At the current stage of research, the two bacteriocins that make up this group are colibacterin and microtoxin, and they differ mainly by their molecular mass [7,8]. However, there is a third type of gram-negative bacteriocin, but it has not

been fully described, so it is also called the pseudo-third type[9].

Colicins are a class of bacteriocins that generally have a molecular weight of 30-80kDa and are usually produced by E. coli strains containing the original plasmid of E. coli. Many studies have proposed that these bacteriocins can be further divided into two subclasses, based on the type of plasmid from which E. coli originates, the first subclass is colicins, which is produced by E. coli, and the other subclass includes colicins, which is produced by other members of the Enterobacteriaceae family [7,10]; However, not all authors have adopted this segmentation approach.

Microcins are a class of bacteriocins containing low molecular weights, typically 1-10 kDa, with a high degree of stability, and the molecular structure of this bacteriocin is active over a wide pH range, the molecular structure of this bacteriocin is not very sensitive to pepsin (which is a very idealized feature of the microbial system and the human digestive system), and remains active under temperature changes and a stable molecular structure [10].

2.2.3 Mechanism of Synthesis of Bacteriocins

Research has consistently demonstrated that bacteriocin synthesis and secretion predominantly occur during the mid-logarithmic phase of bacterial growth, with secretion escalating in tandem with bacterial population density, culminating in a peak secretion rate during the initial stable growth phase. Typically, genes that encode active bacteriocins reside within operon clusters, embedded in genomic DNA, plasmids, or alternative mobile genetic elements. The transcriptional activation of these operons necessitates the presence of autoinducer peptides to elicit expression [11]. The regulation of operon expression is frequently governed by a two-component regulatory system, though, in select instances, it may be orchestrated by a more complex three-component regulatory framework [12].

In this subsection, bacteriocens are mainly divided into two main parts: Gram-positive bacteriocins and Gram-positive bacteriocins. Among them, the gram-positive bacteriocins are mainly lactic acid streptococcins, while the gram-negative bacteriocins are mainly colincins and microcins. and the main synthesis mechanism of bacteriocins. Many studies are now underway for the discovery of new bacteriocins.

3. Mechanism of Action of Bacteriocins

In many studies, researchers have demonstrated some of the effects of a bacteriocin, but the mechanism of action of the found bacteriocin is not well understood, such as: Gosselin, a novel cyclic bacteriocin isolated and purified by Felipe et al. from strain APC 3967, exhibits potent inhibitory activity against Gram-positive foodborne pathogens [13], yet its precise mechanism of action remains elusive. Conversely, several mechanisms of action for bacteriocins have been discerned and exhaustively characterized, with LAB-derived bacteriocins, colloquially known as lacticins, constituting a notable taxonomic example wherein their mechanisms are well-documented [8].

3.1 LAB-Bacteriocins

This bacteriocin is a gram-positive bacteriocin; In this group, one of the most common and well-known types is lanolin antibiotics (the first type).

Lanolin antibiotics have demonstrated two different mechanisms to inhibit bacterial growth, mainly for two cleavage functions: the first is the synthesis that can disrupt the bacterial cell wall; The second is the formation of pores.

3.1.1 Disruption of cell wall synthesis

In this mechanism, many lanolin antibiotics show their antibiotic activity by inhibiting cell wall synthesis in two main ways: the first is binding to cell wall precursor lipid II, thereby inhibiting the final peptidoglycan biosynthesis reaction, resulting in the termination of cell wall synthesis [11], according to the researchers, Mersacidin is a cyclic B-type lanolin thiobacterin produced by Bacillus subtilis , which can interact with the disaccharide pyrophosphate group on lipid II., thereby interfering with transglycosylation and inhibiting the synthesis of peptidoglycan ; The second mechanism is to block the addition of glucose and D-alanine to the precursors of cell wall molecules, thereby inhibiting the synthesis of peptidoglycan; Similar to the first mechanism, which primarily prevents the synthesis of peptidoglycan, this mechanism is also dependent on the availability of lipid II [2].

3.1.2 Pore formation

In this mechanism, lanolin antibiotics can attack the integrity of the cell membrane and thus cause cell death. In this mechanism, two models are currently proposed, one is the „barrel-plate model“ and the other is the „wedge model“. In the „barrel-plate model“, Nisin can rely on this model to inhibit bacterial growth, and the process is mainly as follows: first, the C-terminal region of Nisin binds to phospholipids with anions through electrostatic interactions; Subsequently, the N-terminal portion of Nisin binds to peptidoglycan precursor lipid II. and inserts into the membrane; Finally, the C-terminus of Nisin crosses the cell membrane, switching the peptide to a transmembrane orientation. In the „barrel-plate model“, Nisin is inverted across the membrane to form a circular arrangement at

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the formed holes, which disrupts the integrity of the cell membrane. In the case of the „wedge model“, when there is a high transmembrane potential, the Nisin molecule may change its orientation relative to the membrane plane, bending the lipid surface and forming a wedge-like hole [14,15]. For this mechanism, it is also necessary to participate in the co-participation of lipid II.

3.2 Colicins

There is a mechanism of action that is Gram-negative bacteriocin is also well discovered, the bacteriocin produced by Escherichia coli and other Enterobacteriaceae Colicin, which can be used exclusively to eliminate Gram-negative bacteria. Normally, bacterial cells do not produce colicin, but under the influence of various DNA damaging agents (such as ultraviolet light) and environmental factors, the amount of coli bacteria begins to increase due to nutrient deficiencies or increased bacterial population density. Agents that cause DNA damage or stress cause an “SOS response” that activates the RecA protease and ultimately leads to the self-cleavage inactivation of the inhibitor of E. coli synthesis, allowing transcription of the E. coli operon [21] to produce large amounts of E. coli.

Escherichin consists of three distinct domains: the first is involved in the recognition of specific receptors and binds to specific receptors on the cell surface; The second is the domain involved in translocation, which is for the translocation of E. coli molecules through the cell envelope; The last domain is the one that participates in the lethal activity, leading to the death of the bacteria [15]. And E. coli also has three different mechanisms to kill target cells:

1) Formation of voltage-dependent channels on the inner membrane of the target cell.

2) By influencing the action of nucleases within the cytoplasm.

3) By inhibiting the synthesis of peptidoglycan [15].

Another noteworthy instance involves enzymatically potent colicins, which function as hydrolases or transferases specifically targeting phosphodiester bonds within the DNA or RNA of host cells. Escherichia coli employs the Ton or Tol translocation systems to infiltrate target cells, traversing the inner membrane and gaining access to the cytoplasm, where they exert their lethal enzymatic activities [15].

In this subsection, the main mechanisms of action of two bacteriocins, Gram-positive bacteriocins, and Gram-negative bacteriocins, are introduced. The mechanism of action of gram-positive bacteriocins lanolin antibiotics is mainly to destroy the formation of cell walls and the formation of pores. How to synthesize large quantities of colicins, as well as the three processes in which colicins act, are described in the mechanism of action of gram-negative bacteriocins.

4. Applications of Bacteriocins

4.1 Preservative Agents

Food spoilage represents a significant waste of valuable resources, and the consumption of spoiled food by humans or animals poses a serious health risk. In recent years, to combat food spoilage, the incorporation of chemical preservatives into food products has been prevalent. While these additives, within their recommended dosage, generally pose no apparent harm to humans, their misuse can result in adverse health effects. Furthermore, chemical preservatives are inherently recalcitrant to degradation, thereby contributing to environmental pollution.

However, in the realm of fermented foods, non-pathogenic bacterial consortia, notably LAB, play a pivotal role in enhancing product shelf life. LAB constitute a class of probiotics with extensive applications in food industries, and their bacteriocins are equally valued. Among these, nisin stands as a notable success story, being the sole bacteriocin approved by the Joint FAO/WHO Expert Committee on Food Additives for use as a food preservative [5].

In the context of dairy safety, Listeria monocytogenes and Staphylococcus aureus pose significant threats. The incorporation of nisin into cheese formulations has demonstrated remarkable efficacy in inhibiting the growth of L. monocytogenes while also facilitating the reduction of edible salts and phosphates [16]. Additionally, LAB-derived bacteriocins have found applications in meat processing, where the direct addition of LAB as starter cultures effectively suppresses the proliferation of pathogenic microorganisms [5].

For example, Lactobacillus pentose MS031 isolated from kimchi, a traditional fermented food in Sichuan, China, can be applied to fresh-cut fruit mixtures, reducing mononuclear hyperplastic lactobacillus by 96.3% and controlling levels that cannot be detected by Salmonella and Escherichia coli, which is still in the research stage [17]. The application of bacteriocins to preserve freshly cut products is still under investigation.

4.2 For the Regulatory Function of the Microbiome

A pivotal and well-documented application of bacteriocins lies in their capacity to meticulously modulate the microbiome. This modulation essentially aims to bolster health-promoting microbial populations while diminishing those implicated in disease pathogenesis. Within con-

temporary medical practices, antibiotics play a vital role in combating and safeguarding against infectious agents; however, their imprecise nature can inadvertently compromise the essential gut microbiota [18], exemplified by their potential to diminish short-chain fatty acid production and disrupt intestinal microbiota balance [19].

As scientific investigations into bacteriocins progress, it has been conclusively demonstrated that they can serve as efficacious alternatives to traditional antimicrobials, potentially offering a narrower therapeutic window targeted towards specific microbial genera and species. This targeted approach allows bacteriocins to precisely curb pathogenic growth while preserving the integrity of benign symbionts. Although certain bacteriocins may exhibit broad-spectrum antibacterial activity in comparison to antibiotics, they exert subtle yet significant shifts in microbial composition. Notably, certain Class II bacteriocins, such as penicillin CD produced by Bacillus thuringiensis, have been observed to selectively target and reduce specific microbial groups, like Clostridium, in fecal fermentation models without altering the overall gut microbiota composition [18]. This precise modulation underscores the potential of bacteriocins as a refined tool in microbiome management.

4.3 Application in Medicine

While bacteriocins have exhibited robust antibacterial and preservative capabilities in food applications, recent investigations have underscored their exceptional performance in select medical domains [20]. In the realm of anticancer therapy, while conventional chemotherapy is efficacious against cancer, it often incurs adverse effects owing to its non-specific targeting of healthy cells. Notably, bacteriocins have demonstrated high selectivity and minimal toxicity, thereby emerging as promising candidates for anticancer drugs [20]. This heightened selectivity stems primarily from the ample negative charge on cancer cell surfaces, which avidly binds to the positively charged bacteriocins, fostering a robust electrostatic interaction conducive to effective complexation [21].

Moreover, bacteriocins may find application as antiviral agents. For instance, Radja Teiar et al. examined Enterocin DD14, which inhibited the replication of enveloped viruses. Remarkably, when Enterocin DD14 was combined with lacticaseicin 30 at low concentrations, they synergistically exhibited superior inhibition of HSV-1 (a subtype of herpes simplex virus) [22].

In this subsection, the application of bacteriocin in food preservation, the function of microbiome regulation and the potential application in medicine are mainly introduced.

5. Conclusion

Bacteriocins, as short peptides synthesized by specific bacterial strains, are categorically distinct within the contemporary research paradigm, primarily bifurcated into gram-positive and gram-negative bacteriocins. Most of these biomolecules are extracted and purified from fermented food matrices, exemplified by yogurt and Sichuan Paocai, among others. Notably, the mechanistic underpinnings of various bacteriocins diverge significantly, with contemporary studies predominantly presenting outcomes about their activity, while the precise modus operandi remains elusive, thereby constituting a pivotal research avenue.

Currently, bacteriocins are in an epoch of vigorous development, showcasing their multifaceted utility and demonstrated efficacy across diverse domains, notably the food industry, pharmaceutical sector, medical applications, and biological research. Predominantly, these compounds exhibit bacteriostatic and antimicrobial properties, with lactic acid streptococci standing as the sole entity harnessed for industrial-scale production. Furthermore, bacteriocins harbor immense potential as promising candidates in the combat against drug-resistant microbial strains.

Future endeavors must emphasize the prudent utilization of bacteriocins, mitigating the risks associated with their misuse, and optimizing their societal and scientific worth. This underscores the paramount importance of balanced stewardship in advancing the research and application of bacteriocins, ensuring a harmonious progression of this promising field.

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