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Bacteriocin Production from *Staphylococcus Aureus*: Review Article

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Abstract

A significant pathogen that affects both humans and animals is linked to a variety of illnesses. Treatment for pathogenic strains that are resistant to nearly all existing medicines is a therapeutic problem due to the advent of antibiotic resistance. Globally, developing new antimicrobial strategies is currently of utmost importance. The most varied class of antimicrobial peptides produced by bacteria are called bacteriocins. Bacteriocins are peptides that are synthesized ribosomally and exhibit strong antibacterial activity, typically against bacteria that share phylogenetic relationships with the strain that produces them. Several bacteriocins that exhibit inhibitory activity against harmful bacteria both in vitro and in vivo have been identified from commensal coagulase-negative staphylococci. These bacteriocins are excellent candidates for new therapeutic antimicrobials due to their unique modes of action, effectiveness against bacteria resistant to antibiotics, and capacity to target the production of biofilms. The identification and classification of bacteriocins will progress with the application of genome-mining technologies. The potential of staphylococcal-derived antimicrobial peptides as cutting-edge therapies for infectious strains is covered in this article.

Keywords: Bacteriocins; Antimicrobial Peptides; *Staphylococcus Aureus*; Pahogens

Abbreviations

ET: Exfoliative Toxin; ORFs*:* Open Reading Frames; HPLC: High-Performance Liquid Chromatography; BHI: Brain-Heart Infusion.

Introduction

Bacteriocins are proteins or protein complexes that function biologically by killing bacteria in species that are often closely related. Although variations in antimicrobial activity cannot be linked to specific amino acids or amino acid sequences, bacteriocins fluctuate significantly in molecular weight, isoelectric point (pI), and quantity of specific groups of amino acids. The majority of low molecular weight bacteriocins have cationic properties at low pH values, hence augmenting their antibacterial efficacy. Studies on the spectrum of activity, mode of action, impact of heat, pH, proteolytic enzymes, salt, and detergents on bacteriocin activity, as well as the determination of molecular mass, amino acid composition and sequence, and genetic organization of bacteriocin production and secretion are all part of the characterization of bacteriocins [1].

These proteins are made by a wide variety of microorganisms. Within the family Micrococcaceae, the genus Staphylococcus comprises Gram + non-spore forming cocci that are commonly present in the typical human skin and nasal cavity microbiota. Staphylococcin, also known as Bacteriocin, is a proteinaceous inhibitor that is generated by Staphylococcus aureus. Because staphylococcins may inhibit a wide variety of bacterial species, including a number of bacterial diseases, they may have useful uses in the food business as biopreservatives and as a potential substitute for antibiotics in medicine [2].

Classification

1. Class I (Lantibiotic (Staphylococcin C55 alpha, Staphylococcin C55 beta))
2. Class II (Aureocin A53, Aureocin 70, Aureocin 4185)
- Cyclic Bacteriocin
- Other Bacteriocin
3. Class III (Lysostaphin) produced from *Staphylococcus Simulans*

Lantibiotic

A component of bacteriocin is lanthionine, also known as lantibiotic, which undergoes post-translational modification to become methyl lanthionine. A bacteriocin from class I bacteriocins, C55, was generated by S. aureus. It is composed of two peptides, C55a and C55b, and contains lanthionine. According to Kawada-Matsuo, et al. C55 has antibacterial action against S. epidermidis but not against Micrococcus luteus, Lactococcus lactis, or S. aureus [3]. According to O'Connor EB, et al. C55 exhibited significant similarity to bacteriocin lacticin 3147 generated by Lactobacillus lactis. Because the pETB plasmid contains genes encoding bacteriocin, it has been shown that ETB-positive S. aureus strains produce the lantibiotic C55 [4].

Staphylococcus aureus produces exfoliative toxin (ET), which is closely linked to the beginning of bullous impetigo. There are three identified ETs: ETA, ETB, and ETD. According to Deegan LH, et al. this group of S. aureus strains produces phage II bacteriocin extensively, which is the adopted prototype [5,6]. The strain S. aureus C55 contains its genetic determinants on a 32 kb plasmid; nevertheless, its genetic determinants have also been found on a 37 kb plasmid in S. aureus U0007 [7] and on a 38 kb pETB plasmid from the clinical isolate S. aureus TY4 [8,9]. Fascinatingly, the bacteriocin structural genes are connected to an exfoliative toxin B determinant in every instance. This exotoxin is linked to human skin illnesses [10]. Staphylococcin C55α and C55β, structural peptides with molecular weights of 2993 and 3339, respectively, are needed in equimolar levels to work in concert and have an antibacterial effect on strains of S. aureus and Micrococcus luteus but not S. epidermidis. The mechanism of action of this partially purified substance, which was discovered to cause cell death, was pore development in the cytoplasmic membrane and extensive inhibition of macromolecular production [7].

Class II

Only a tiny percentage of Staphylococcus aureus strains generate a material known as "aureocin."

Aureocin A53: Bovine mastitis is one of the cattle diseases that strain A53 may indicate new antimicrobial peptides that might be used to treat and prevent.

Staphylococcus aureus A53 is the producer of aureocin A53. It is active against Listeria monocytogenes and is encoded on the 10.4 kb plasmid pRJ9. A 51-residue peptide with ten lysine and five tryptophan residues, aureocin A53 is highly cationic. Using reverse-phase chromatography, cation exchange, and hydrophobic interaction, aureocin A53 was homogenizedly purified. A molecular mass of 6012.5 Da was obtained using MALDI-TOF mass spectrometry. A conventional bacteriocin leader sequence or sec-dependent signal peptide is not present in the synthesis or secretion of aureocin A53, as evidenced by the mass increment caused by an N-formylmethionine residue. None of the open reading frames located near the structural gene aucA exhibited similarities to genes commonly seen in bacteriocin gene clusters, according to the whole sequence of pRJ9. It has been discovered that the synthesis of aureocin A53 does not involve the presence of any transporters, modifying enzymes, or proteases. Aureocin A53 differs from other peptide bacteriocins in addition to having a distinct, stiff structure in aqueous solution and exceptional protease stability [11].

Aureocin A70: *Aureocin A70 is a heat-stable bacteriocin that is produced by Staphylococcus aureus A70. Listeria monocytogenes is the target of aureocin A70, which is expressed on the plasmid pRJ6, an 8 kb plasmid that is mobilizable. HindIII-A and B segments of pRJ6 were linked to aureocin A70 production and immunity, according to transposition mutagenesis and gene cloning experiments. As a result, concatenated DNA sequencing was used to sequence a 6332 bp section of the plasmid that included both of these fragments. Three transcriptional units were identified by genetic and DNA sequence analysis, and they seem to be involved in the activity of bacteriocin The single gene aurT, which produces a protein resembling an ATP-dependent transporter like those involved in lantibiotic export, is found in the first transcriptional unit. Both the synthesis of aureocin A70 and the mobilization of pRJ6 seem to depend on AurT. Two open reading frames (ORFs) make up the second putative operon; the first gene, orfA, is predicted to encode a protein that resembles tiny repressor proteins that*

are present in some Archaea but whose exact function is yet unknown. The second gene, orfB, produces a protein with a Bierbaum G8 amino acid residue that is required for self-defense against bacteriocin. It has several traits with proteins linked to immunity, including a high pI and hydrophobicity profile. The operon aurABCD has four more genes. Four similar peptides with a pI of 9.85 to 10.04, short (30–31 amino acid residues), and high hydrophobicity are encoded by aurABCD. In addition, these peptides lack cysteine residue and have a significant concentration of minor amino acid residues, such as glycine and alanine. Operation aurABCD contains Tn917-lac insertional alterations that impacted aureocin A70 activity. The production, expression, and excretion of all four peptides encoded by the aurABCD operon occur without post-translational changes, according to mass spectrometry analysis of purified bacteriocin preparations. Aureocin A70, then, is a multi-peptide non-lantibiotic bacteriocin that travels unprocessed [12].

Aureocins 4185: The strain of Staphylococcus aureus 4185 that was isolated from bovine mastitis produced the bacteriocins that were purified and partially described in the current investigation. Antimicrobial activity was recovered with 40% and 80% isopropanol following purification by ammonium sulfate precipitation, cation-exchange chromatography, and five runs of high-performance liquid chromatography (HPLC). This suggests that S. aureus 4185 produces multiple antimicrobial peptides, known as aureocins 4185. [Peptides A (2,305.3 +/-1.5 Da), B (2,327.3 +/-1.5 Da), and C (3,005.5 +/-1.5 Da) were found to elute with 40% isopropanol, while peptides D (6,4Bierbaum G.5 +/-1.5 Da) and E (12,834.5 +/-1.5 Da) eluted with 80% isopropanol, according to mass spectrometry analysis. Using matrix-assisted laser desorption/ionization time of flight (MALDI-TOF)/TOF mass spectrometry analyses, only four small peptide sequences were obtained, despite the detection of five peptides: SLLEQFTGK (eluted with 40% isopropanol), ALLYDER, NNTSHNLPLGWFNVK, and NNLAQGTFNATK (eluted with 80% isopropanol). SLLEQFTGK and ALLYDER sequences demonstrated identity with putative peptides of unclear function. The sequences NNLAQGTFNATK and NNTSHNLPLGWFNVK exhibited similarities to a section of a staphylococcal autolysin precursor.

The strain 4185 supernatant's antibacterial activity was found to be resistant to heat treatment at 65°C; however, treatment at 80°C totally eliminated the antimicrobial activity. According to Ceotto H, et al. there was a significant bacteriolytic activity of the concentrated supernatant containing aureocins 4185 against Micrococcus luteus ATCC 4698 [13].

Cyclic bateriocin of *staphylococcus aureus*: It has previously been demonstrated that Staphylococcus aureus 4185 produces two or more bacteriocins. PRJ101 is encoding one of them. In order to identify the gene cluster expressing bacteriocin, a ~9160 kb section of pRJ101 underwent sequencing. One of these appears to be a cyclic peptide, a kind of bacteriocin that the genus Staphylococcus initially reported. Plasmid pRJ101 is the one that encodes aureocyclicin 4185, the novel staphylococcin. Nevertheless, Aureocycli cin 4185 could not be found in the pRJ101 host strain's culture supernatant, indicating that the cells only produce a small amount of this bacteriocin [14].

Other bacteriocin produced from *staphylococcus aureus*: A study was conducted on the chemical, physical, and biological aspects of staphylococcin (414), a bacteriocin generated by a strain of Staphylococcus aureus. After the cells split, a sizable amount of staphylococcin is released, which can be separated using column chromatography and differential centrifugation. It seems to be a lipoprotein-carbohydrate complex with a molecular weight greater than 200,000 in its natural state. Sodium dodecyl sulfate has the ability to split the complex into smaller, still-active subunits. The bacteriocin's overall chemical and physical characteristics are quite similar to those of several cell membrane preparations. Unlike lysostaphin, staphylococcin (414), is not a lytic enzyme and exhibits a different range of activity. It inhibits several other genera but not any gram-negative bacteria, like other bacteriocins from gram-positive microorganisms [15].

Out of 200 staphylococci isolates of an animal origin, three strains that produce bacteriocin were chosen for analysis, including Staphylococcus aureus 462. The activity of these bacteriocins is selective; only specific strains of S. aureus and other gram-positive species are inhibited in growth, not gram-negative organisms. The supernatant fluid of broth cultures did not contain any substantial amounts of Staphylococcin 462, nor did it release the bacteria into the suspending liquid upon manually disrupting the cells. But most of the activity was released after the cells were extracted using 7 M urea. The substance was purified by preparative electrophoresis on polyacrylamide gels in the presence of sodium dodecyl sulfate and gel permeation chromatography using Sephadex G-200. According to chemical examination, the substance was around 90% protein and 3% fat. It was determined that staphylococcin 462 that was dissociated by sodium dodecyl sulfate had a molecular weight of almost 9,000.

Staphylococcus Aureus AB188

It has been discovered that (a clinical isolate from wound pus) produces bacteriocins and/or bacteriocin-like inhibitory substance(s), which are being provisionally dubbed staphylococcin Bac188. It exhibits broad-spectrum

activity against a wide range of bacteria, including dermatophytes such as Microsporum canis, Microsporum gypseum, Trichophyton mentagrophytes, Trichophyton longi, and Trichophyton rubrum, as well as a variety of Gram-positive and Gram-negative bacteria like E. coli, S. typhi, and S. dysenteriae. Remarkably, staphylococcin Bac188 had strong efficacy against numerous clinical isolates of Mycobacterium tuberculosis as well. Over a broad pH range (pH 2–14), Staphylococcin Bac188 demonstrated broad thermostability and remained stable. Additionally, it exhibited resistance to treatment with lipase, lysozyme, catalase, and chloroform; nonetheless, it exhibited susceptibility to proteinase K, trypsin, and α-chymotrypsin, indicating that it was proteinaceous. Bacteriocidal effects of Staphylococcin Bac188 were seen on the sensitive strain of S. aureus, E. coli, and S. typhi, indicating a possible identical mode of action for both Gram-positive and Gram-negative organisms. S. aureus AB188 has antibacterial, antidermatophytic, and antimycobacterial actions that are correlated with the synthesis of a bacteriocin or bacteriocin-like inhibitory compound, which shares characteristics with other previously reported staphylococcins. This is the first article that we are aware of that describes the broad range of prospective clinical applications of a bacteriocin and/or bacteriocin-like inhibitory chemical produced by S. aureus AB188. It suggests that more research be done in order to potentially develop therapeutic applications [16].

Growth Conditions

The study examined the impact of growth conditions on the bacteriocin (aureocin) synthesis of five distinct strains of Staphylococcus aureus. These aureocins can suppress significant human and animal diseases and have a wide range of activities. Large inhibitory zones were created by all strains upon the indicator strain when they were cultured in rich media, including N2GT, 2 • YT, and brain-heart infusion (BHI). The initial pH of the medium (6.0–8.0) had no effect on the formation of bacteriocin. There was a noticeable decrease in the generation of bacteriocin at a lower temperature (280 C). Two related bacteriocins, aureocins MB92 and 146L, were significantly reduced and aureocins A53 and 215FN, marginally increased, throughout the producers' anaerobic incubation. Aureocins MB92 and 215FN production appeared to be eliminated in conditions containing more than 2.5 g and 3.5 g of NaCl per 100 milliliter, respectively. All examined NaCl concentrations (0.5–7.5 g/100 ml) showed production of the remaining aureocins; however, the greatest inhibition zones were seen in medium containing up to 1.5 g (for aureocins A70 and 146L) or 2.5 g NaCl/100 ml (for aureocin A53). The culture supernatant did not contain Aureocin 215FN. These kinds of characteristics matter for evaluating the possible biotechnological uses of these compounds and when evaluating novel isolates of S. aureus for the generation of bacteriocin [17].

Applications of Bacteriocin

- Aureocin A70, a bacteriocin that inhibits food-borne pathogen strains of Listeria monocytogenes
- In animal models, such as mice, hens, and pigs, the amount of pathogens or the makeup of the gut microbiota can be altered by purified bacteriocins or probiotics that produce them.
- Aureocins 4185 showed potential for use in food preservation by demonstrating antagonistic action against significant foodborne pathogens, such as Listeria monocytogenes.
- Certain studies suggest that bacteriocins exhibit antitumor cell activity in cancer therapy. Given that bacteriocins may be given to food in a natural and legal way, they could be a good option for an anti-tumor medication.
- A few bacteriocins generated by Gram-positive bacteria are employed in bacterial consortia as communication molecules and as chemical mediators in quorum sensing [18,19]. One well-researched mechanism for controlling the bacteriocin gene is quorum sensing. According to Wang, et al. (20Bierbaum G), quorum sensing is a cell-density dependent regulatory system in which cell-to-cell communication is mediated by an autoinducing signal molecule. Through this mechanism, every bacterial cell detects the quantity of cells belonging to the same species or strain and modifies the time of specific gene expression [20].
- Animal farming poses the same challenges to aquatic cultures. A variety of bacteria, some of which are dangerous, are constantly exposed to them [21]. Numerous attempts were made to stop and manage this problem. The use of bacteriocin-producing bacteria, or BPB, is an alternate strategy for disease control in aquaculture [22]. It suggests that the interaction between the microbiota, including probiotics, and the host is not restricted to the intestinal tract and that these bacteria can be used as probiotics because in aquaculture, aquatic animals and microorganisms share the same ecosystem in the aquatic environment [23]. According to numerous studies, administering BPB as a probiotic can improve water quality, boost the immune system of the host species, reduce the growth of competitively pathogenic bacteria by producing compounds that inhibit their growth, and improve the nutrition of the host species by producing extra digestive enzymes [24,25].
- Food preservation makes extensive use of bacteriocins. There has been much research done on the use of bacteriocins in the food sector, particularly with regard to dairy, egg, vegetable, and meat products. However, a variety of preservation techniques have been employed

to stop food spoiling and food poisoning. These methods include addition of preservatives (antibiotics, organic compounds such as propionate, sorbate, benzoate, lactate, and acetate), lowering of pH and water activity (acidification, dehydration), and thermal treatment (pasteurization, heating sterilization). Even though these techniques have shown to be quite effective, there is a growing market for natural, microbiologically safe products that offer significant health advantages to customers. Bacteriocins can be added to food in either a refined or unrefined form, or they can be produced by fermenting a product and adding it to another strain of bacteria that produces bacteriocins.

- Furthermore, bacteriocins can be coupled with other antimicrobial substances to further boost the inactivation of germs, such as sodium acetate and sodium lactate. Additionally, by speeding up the process of proteolysis or preventing the gas blowing defect in cheese, bacteriocins can enhance the sensory qualities and quality of food.
- Bioactive packaging, a technique that can shield food from outside pollutants, is another use for bacteriocins. For example, microbial growth on the surface of chilled food often initiates food spoiling, which supports the appealing use of bacteriocins in conjunction with packaging to enhance food safety and self-life [25].
- The preparation of bioactive packaging involves either adding a sachet containing bacteriocin to the packaged food, which will be released when the food product is stored, or directly immobilizing the bacteriocin to the food packaging. Because antimicrobial activity may be lost or reduced due to bacteriocins being inactivated by food components or dilution below active concentration due to migration into the foods, the gradual release of bacteriocins from a packaging film on the food surface may have an advantage over dipping and spraying foods with bacteriocins [26]. There are various techniques for making packaging films that include bacteriocins Table 1.

S. no.	Bacteriocin	Plasmid Encoded	Molecular Mass (Da)	Effective against Microorganisms
1	Lantibiotic			
	C55 alpha	32-38 kb (pETB)	3339	*Micrococcus luteus*
	C55 beta		2993	
2	*Aureocin 70*	8.0kbpRJ6		Listeria monocytogenes
3	*Aureocin A53*	10.4 kb(pRJ 9)	6012.5	
4	*Aureocin 4185*	pRJ101		*Micrococcus luteus*
5	*Cyclic Aureocin 4185*	pRJ101		
6	Staphylococcin 414		200,000	
7	Staphylococcin 462		9,000	
8	Staphylococcin AB188			B.subtilis, S.aureus, E.faecalis, E.coli, S.typhi and S.dysenteriae

Table 1*: Characteristics of different types of Bacteriocins.*

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