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Review

BLIS Fingerprinting as a Tool to Investigate the Distribution and Significance of Bacteriocin Production and Immunity in *Streptococcus pyogenes* and *Streptococcus salivarius*

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Abstract: The study of bacteriocins has significantly enhanced our understanding of microbial interactions, notably within the genus *Streptococcus*. Among the most functionally diverse and clinically relevant bacteriocins are those belonging to the lantibiotic class, which exhibit potent antimicrobial properties and are central to the competitive dynamics of streptococcal species. This review focuses on the discovery and characterization of bacteriocins produced by *Streptococcus pyogenes* and *Streptococcus salivarius*, emphasizing their biological significance within their exclusive human host. A cornerstone of these studies has been the development and application of the pioneer agar culture-based bacteriocin detection methodology, known as streptococcal bacteriocin fingerprinting. This approach has proven invaluable for the initial detection and differentiation of a wide array of bacteriocin-like inhibitory substances (BLIS) in streptococcal populations. A central theme of this review is the diverse biological roles of lantibiotics in *S. pyogenes* and *S. salivarius*, particularly in relation to microbial competition, colonization dynamics, and host interactions. The expression of lantibiotic determinants provides distinct advantages to the producing strain, including enhanced niche establishment and the ability to suppress competing microbes. Furthermore, the presence of specific lantibiotic immunity mechanisms safeguards the producer from self-inhibition and potential antagonism from closely related competitors. In *S. pyogenes*, lantibiotic production has been implicated in virulence modulation, raising important questions about its role in pathogenicity and host immune evasion. Conversely, *S. salivarius*, a prominent commensal and probiotic candidate species, utilizes its lantibiotic arsenal to confer colonization benefits and mediate beneficial interactions, especially within the oral and upper respiratory tract microbiomes. The implications of in situ lantibiotic expression extend beyond microbial ecology, presenting opportunities for innovative probiotic and therapeutic applications. The potential for harnessing bacteriocin-producing streptococci in antimicrobial interventions, particularly in combating antibiotic-resistant pathogens, underscores the translational relevance of these findings. This review integrates historical and contemporary perspectives on streptococcal bacteriocin research, providing insights into future avenues for leveraging these bioactive peptides in clinical and biotechnological contexts.

Keywords: BLIS fingerprinting; bacteriocin; lantibiotic; *Streptococcus salivarius*; *Streptococcus pyogenes*; salivaricin; antimicrobial; bacteriocin immunity; microbial competition; virulence modulation

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1. Principal Objectives

1. Provide a contemporary overview of the expression of Class I and Class III bacteriocin loci in *S. pyogenes* and *S. salivarius*, with an emphasis on the distribution and potential roles of the expressed products of the Class I (viz., lantibiotic) structural and immunity genes.
2. Showcase original findings from the now "classical" agar culture-based streptococcal bacteriocin fingerprinting method, focusing on its contributions to the discovery of novel bacteriocins and its role in understanding microbial competition and niche adaptation.
3. Application of the fingerprinting methodology in combination with PCR to evaluate the distribution of bacteriocin-like inhibitory substance (BLIS) production and/or the expression of specific BLIS immunity in an extensive collection of reference strains of *S. pyogenes* representing more than 100 different M-protein or emm types.
4. Discuss some of the broader implications of lantibiotic systems for bacterial survival and virulence, as well as for human health and therapeutic innovation. Discussion points include the following:
    a. The widespread expression of salivaricin A immunity determinants in *S. pyogenes*, whereas functional salivaricin A production appears essentially limited to serotype M-type 4 strains.
    b. The putative absence of salivaricin B production in *S. pyogenes*.
    c. The apparently universal sensitivity of *S. pyogenes* to the salivaricin A/salivaricin B-producing probiotic strain *S. salivarius* BLIS K12.

By combining reflections on pioneering achievements with insights from recent discoveries, this review highlights the dual importance of streptococcal bacteriocins in fundamental microbiology and translational applications. It focuses particularly upon the relevance of lantibiotic-producing streptococci both as tools for microbial competition in probiotic applications and as potential assets for future antimicrobial development.

2. Introduction

The first author's scientific journey began with a deep fascination for bacteriocins as specifically targeted intra-generic weaponry, potentially providing the producer bacterium with a survival edge in highly competitive microbial ecosystems. This interest was first fostered during studies of bacteriocin production by *Pseudomonas aeruginosa*, where an agar-based pyocin fingerprinting method was developed for strain typing purposes to assist with monitoring the transmission of *Ps. aeruginosa* in a hospital setting [1,2]. This methodology provided a robust basis for strain differentiation and epidemiological tracking. Importantly, it also hinted at the broader ecological significance of bacteriocins as mediators of microbial competition and niche dominance.

The subsequent discovery that *Streptococcus pyogenes* also produces bacteriocins led to a research career focused on increasing our understanding of the "raison d'être" for these molecules, enabled initially by the design and application of a technologically simple streptococcal bacteriocin fingerprinting method—mirroring the basic principles already established for pyocin fingerprinting [3]. This adaptation required tailored approaches to accommodate the diverse optimal growth conditions for streptococcal bacteriocin expression, the heterogeneous molecular characteristics of the bacteriocins, and the sometimes strain-specific inhibitory spectra of streptococcal bacteriocins. The outcome was a reliable agar-based method for detecting and categorizing bacteriocin production and immunity patterns in strains of a wide variety of streptococcal and several non-streptococcal species. Although now considered "technologically old-fashioned", this tool has provided a robust framework for exploring the natural variation in BLIS activities across numerous bacterial

isolates, leading to the discovery of a heterogeneous collection of Class I (lantibiotics), Class II (small unmodified peptides), Class III (large > 10 kDa), and Class IV (circular) bacteriocins [4,5] and laying the foundation for subsequent discoveries. Structural analyses of several class I lantibiotics from streptococci are described by Barbour et al. [6].

It soon became clear that streptococci produce a wide diversity of bacteriocins, including but not limited to lantibiotics, and that these molecules play critical roles in microbial interactions and host adaptation. This realization initiated a program of exploration, culminating in the discovery of a series of bacteriocins, the progenitor being the nisin-like lantibiotic streptococcin A-FF22 (SA-FF22) [7]. The implications of these molecules for microbial ecology and human health continue to attract the attention of researchers and health professionals.

This review explores several key questions in relationship to these bacteriocins:

- How do bacteriocins and their associated immunity loci influence microbial survival and behavior?
- What roles do these peptides play in shaping host–microbe interactions?
- Can insights from these antimicrobial systems inspire new therapeutic strategies?

Among the streptococci, *S. pyogenes* and *S. salivarius* hold special significance. These two species, while sharing humans as their sole hosts, assume strikingly different ecological and pathogenic profiles within the human ecosystem [8]:

- *S. pyogenes* is a formidable pathogen, responsible for diseases ranging from pharyngitis to severe invasive infections, some with chronic or lethal sequelae. It is an adept colonizer and an uncompromising exploiter of diverse host niches [9].
- *S. salivarius*, on the other hand, is a benign commensal, thriving predominantly in the oral cavity and intestinal tract, where it contributes to microbial homeostasis through its interactions with other microbiota and the immune system [10,11].

Despite their contrasting lifestyles, both species commonly harbor lantibiotic and other bacteriocin loci—genetic blueprints encoding potent antimicrobials and the corresponding specific bacteriocin immunity molecules. Interestingly, some, but not all, of these bacteriocins are produced by members of both of these streptococcal species. In *S. pyogenes*, many (but not all) of the bacteriocin loci appear to be chromosomally located, whereas in *S. salivarius*, mega-plasmids sometimes accommodate multiple bacteriocin loci [12].

By focusing on the interplay between these two streptococcal species, their bacteriocins, and their human host, this review aims to shed light on their intricate relationships and potential applications.

3. The Bacteriocin Fingerprinting Scheme: Its Role in Discovering Lantibiotics and Other Bacteriocins in *S. pyogenes* and *S. salivarius*

The bacteriocin fingerprinting scheme classifies bacterial strains based on both their production of and sensitivity to bacteriocins [3]. By assessing the inhibitory spectrum produced against a set of nine specified indicator strains under standardized conditions in a designated agar medium, a bacteriocin "P-type" (production type) is assigned to the test strain. Conversely, the "S-type" (sensitivity type) identifies the test strain's susceptibility to the bacteriocins produced by nine standard bacteriocin producers. The resulting bacteriocin "fingerprint"—a combination of P-type and S-type—provides an in vitro snapshot of both the offensive and defensive bacteriocin-mediated capabilities of the test strain in microbial interactions. The triplet code designation applied for the derivation of these "fingerprints" has been described by Tagg and Bannister [3]. These fingerprints serve as reproducible phenotypic signatures, enabling comparisons across diverse bacterial collections and advancing our understanding of bacterial interactions and antimicrobial activity within heterogeneous

streptococcal populations. The bacteriocins of Gram-positive bacteria can be classified into four major divisions [13]. (a) Class I post-translationally modified small (<10 kDa) peptides; (b) Class II non-modified small peptides; (c) Class III large (>10 kDa) proteins; and (d) Class IV cyclic peptides. The bacteriocins shown to be produced by the standard producer and indicator strains are listed in Table 1.

Table 1. Bacteriocins produced by the standard indicator and producer strains [4].

Code *	Strain Identity	Bacteriocin Product		Mass (kDa)	Ref.
		Name	Type		
Standard producer strains					
P1	*S. pyogenes* M4	Salivaricin A1	Class I type AII lantibiotic	2.327	[14]
P2	*S. pyogenes* FF22	SA-FF22	Class I type AII lantibiotic	2.794	[15]
P3	*S. agalactiae* 74-628	Nisin U2	Class I type AI lantibiotic	3.029	[16]
P4	*S. dysgalactiae* W2580	Dysgalacticin	Class III type b nonlytic protein	21.5	[17]
P5	*S. pyogenes* M28689	Streptin	Class I type AI lantibiotic	2.424	[18]
P6	*S. constellatus* subsp. *constellatus* T29	Stellalysin	Class III type a lytic protein	29.0	[19]
P7	*S. pyogenes* 71-724	SA-M57	Class III type b nonlytic protein	17.0	[20,21]
P8	*Enterococcus faecalis* T-142	BLIS activity not characterized			
P9	*S. pyogenes* 71-722	BLIS activity not characterized			
Standard indicator strains					
I1	*Micrococcus luteus* T-18	BLIS negative			
I2	*S. pyogenes* FF22	Same as P2			
I3	*S. constellatus* subsp. *constellatus* T29	Same as P6			
I4	*Streptococcus uberis* ATTC 27958	Nisin U	Class I type AI lantibiotic	3.029	[16]
I5	*S. pyogenes* M4	Same as P1			
I6	*Lactococcus lactis* T-21	BLIS activity not characterized			
I7	*S. pyogenes* 71-698	Dysgalacticin	Class III type b nonlytic protein	21.5	
I8	*S. pyogenes* W-1	Streptin	Class I type AI lantibiotic	2.424	
I9	*S. dysgalactiae* subsp. *equisimilis* T-148	BLIS activity not characterized			

* P1–P9 and I1 > I9 are code designations for the standard bacterial producer and indicator strains, respectively.

For bacteriocin fingerprinting to be successful, several technical factors need to be meticulously controlled:

- Enhanced Detection: blood supplementation of the basal nutrient agar medium and incubation at a reduced temperature (32 °C) in a 5% CO_2 in-air atmosphere improved bacteriocin detection.
- Physicochemical Properties: the bacteriocins exhibited diverse properties distinct from those attributable to hydrogen peroxide or pH-based inhibitory effects.
- Solid-phase Assays: some bacteriocins were not produced in liquid media, emphasizing the requirement for solid-phase assays for optimal detection.

The culture medium composition and incubation conditions (temperature, aeration, and time) were important influencers on the detection of bacteriocin production. For instance, the production of the *S. pyogenes* lantibiotic streptin was strongly influenced even by minor batch-to-batch variations in the composition of the commercially supplied Columbia Agar base medium used for the test [22], underlining the subtle factors impacting bacteriocin expression and thereby the competitiveness of the producer strains in situ. Furthermore, isolates of *S. pyogenes* serotype M49 showed markedly increased production

of SA-FF22 in anaerobic conditions [23]. Although conceptually simple, the streptococcal bacteriocin fingerprinting method is remarkably robust, providing a cost-effective and practical means to explore bacterial diversity and microbial interactions. This method has proven invaluable in identifying a diverse array of novel bacteriocins and in deepening our understanding of microbial ecology. It has illuminated the role of bacteriocins as essential mediators of microbial competition and survival. Indeed, many of the foundational insights derived from the application of this technique continue to drive advances in antimicrobial discovery.

Application of bacteriocin fingerprinting has revealed extensive inter-strain variability in the bacteriocinogenicity of *S. pyogenes* and *S. salivarius*, unearthing a broad range of distinct production profiles (P-types) and sensitivity/resistance patterns (S-types). Interestingly, further applications of the fingerprinting methodology have also facilitated the detection of BLIS activities in several non-streptococcal bacteria, including staphylococci ([24,25], enterococci [26], actinomyces [27], and lactobacilli [26]). These findings underscore the complex, inter-generic inhibitory activities of many of the bacteriocins of Gram-positive bacteria [28,29], some of which are now seen as promising candidates for therapeutic applications [30].

3.1. Key Contributions of the Bacteriocin Fingerprinting Methodology

1. Discovery of Novel Lantibiotics and Non-lantibiotic Bacteriocins: further exploration of these discoveries is discussed below.
2. Revealing Immunity Profiles: the fingerprinting method revealed that many strains exhibit immunity to specific bacteriocins, even in the absence of their production of the corresponding bioactive bacteriocin. This was particularly evident in *S. pyogenes*, where immunity to salivaricin A is widespread despite its production being essentially limited to serotype M4 [31]. These findings suggest that immunity determinants might serve functions beyond the conferring of specific bacteriocin resistance, potentially also aiding *S. pyogenes* survival against host-derived cationic antimicrobial peptides. Notably, the widespread immunity to salivaricin A in *S. pyogenes* results in bacteriostasis rather than bacteriocidal effects, making it less likely in situ for *S. salivarius* that produce only salivaricin A to outcompete these salivaricin A-immune *S. pyogenes*. *S. salivarius* strains such as BLIS K12, which produce other bacteriocins (e.g., salivaricin B), on the other hand, appear better equipped to successfully compete with these *S. pyogenes*.
3. Detection of Orphan Patterns: the fingerprinting method has identified a number of unique P-type and S-type patterns indicating the expression of uncharacterized bacteriocin loci and subsequently stimulating ongoing research into the diversity of antimicrobial peptides within the streptococci and other Gram-positive bacteria.
4. Preliminary Profiling of Ecological Impact: the method provides insights into how bacteriocins might influence inter-bacterial dynamics, particularly in specific niches like the oral cavity and pharynx, by assessing both inhibitory activity and susceptibility to bacteriocins.
5. Evolutionary Insights: the method contributes to understanding the evolutionary pressures shaping bacteriocin loci, especially in relationship to the benefits of decoupling bacteriocin production and immunity expression.
6. A Screening Tool for Therapeutic Applications: the fingerprinting approach is an invaluable tool for selecting strains with potential probiotic and therapeutic applications, as exemplified by *S. salivarius* strains BLIS K12 and BLIS M18, which are both now widely used for oral health and other clinical purposes [32].

3.2. Future Directions and Ongoing Relevance

While genomic tools now enable the direct identification of bacteriocin loci [33,34], bacteriocin fingerprinting remains an essential complementary research tool. By linking genetic data to phenotypic expression, it bridges the gap between sequence-based predictions and functional outcomes. Ongoing investigations into uncharacterized P-type patterns in *S. pyogenes* and *S. salivarius* continue to demonstrate the method's value in uncovering novel bacteriocins and expanding our understanding of microbial interactions. The development of BLIS fingerprinting was a key milestone in studying antimicrobial peptides and their role in microbial competition, particularly among the streptococci. One limitation to be aware of when conducting BLIS fingerprinting is that follow-up plasmid curing or gene deletion studies are required to confirm that a specific genetic element is responsible for the bacteriocin resistance and that it is not the result of intrinsic resistance that is an innate characteristic of that bacterial species. This methodology has proven to be a versatile tool, providing insights that have remained relevant to the discovery of novel antimicrobial agents (Table 2).

Table 2. Bacteriocins of streptococci first detected by BLIS fingerprinting.

Source	Lantibiotic	Other BLIS Activity	Comments
S. pyogenes	SA-FF22		Demonstrated to be nisin-like [7]. Structure [35] and mode of action [15] established.
	SA-M49		Closely similar to SA-FF22 [23]. Produced by 11/32 tested serotype M49 and 4 of 8 serotype M52 isolates [36].
		Streptococcin A-M57	Produced by all of 35 tested serotype M57 strains [21]. 2.2 MDa plasmid-encoded [37].
	Salivaricin A		Originally named "the inhibitor" [38]. Produced by all of the 12 tested serotype M4 T4 strains [3]. A total of 63/65 *S. pyogenes* hybridized with a SalA1 structural gene probe [39]. Serotype M4T4 strains of *S. pyogenes* persisted in a carriage state longer than other *S. pyogenes* [31].
	Streptin		P-type 777 activity produced by (a) 35/350 tested *S. pyogenes* [22] and (b) 10/73 M-prototype *S. pyogenes* [36]. Established to be a type A1 lantibiotic [18].
S. uberis			Demonstration of 9 different P-types in 15 strains [40].
	Nisin U		A nisin variant [16].
		Ubericin A	Class IIa bacteriocin [41].
		Uberolysin	Cyclic bacteriocin [42].
S. agalactiae		Streptocin B_1	A 10,000 Da peptide [43].
S. salivarius	"Inhibitors"		Original study describing 6 heterologous "inhibitors" [44].
	Salivaricin A1		Isolated from strain 20P3 [45].
	Salivaricin A2		Isolated from strain K12 [12].
	Salivaricin G32		Homolog of SA-FF22 [46].
	Salivaricin E		Active against *S. mutans* [47]
	Salivaricin 9		The prototype producer (strain 9) was one of six inhibitory strains identified by Dempster and Tagg [44,48].

Table 2. *Cont.*

Source	Lantibiotic	Other BLIS Activity	Comments
S. salivarius	Salivaricin M		Active against *S. mutans* [49].
	Streptococcin sal-P Salivaricin B		Later established to be a homolog of salivaricin B [50]. Isolated from strain K12 and megaplasmid encoded [12].
		Salivaricin SN	Zoocin-like muralytic enzyme [14].
		Salivaricin MPS	Production enhanced in saliva and in an anaerobic atmosphere [44].
Mutans streptococci			P-type differentiation of reference mutans streptococci [51].
S. mutans		Mutacin N	A novel group I mutacin [52]. Genetic basis established [53].
		Mutacin IV and mutacin V	Products of reference strain UA159 [54].
	Mutacin K8		Type A11 lantibiotic [55].
S. rattus	BHT-A	BHT-B	BHT-A is a variant of the two-component lantibiotic, Smb. BHT-B is a non-modified 5195 Da peptide similar to the tryptophan-rich *Staphylococcus aureus* bacteriocin, aureocin A53 [56].
S. gordonii		Streptocins STH_1 and STH_2	Competence-dependent bacteriocins [57].
S. zooepidemicus		Zoocin A	Lysostaphin-like BLIS having extensive bacteriolytic activity against mutans streptococci [26,58,59].
Nutritionally variant streptococci		Bacteriolysin activity	A total of 5/5 *S. adjacens* bacteriolytic for *M. luteus* [60].
S. sanguinis		Sanguinicin K11	Produced by sucrose/bacitracin-tolerant *S. sanguinis*. Has activity against *S. rattus* [61].
Group B and Group C streptococci			BLIS fingerprinting demonstrated 6/120 group B and 9/50 group C were BLIS producers [62].
Group E streptococci			A total of 5 different P-types detected in 12 isolates [63].
Group F streptococcus		Streptococcin F-29 (stellalysin)	Heat-labile anti-*S. pyogenes* activity [64]. Similar to zoocin A [19].
Group G *streptococcus*, viz., *S. dysgalactiae*		Streptococcin G-2580 and Dysgalacticin	A total of 12/30 strains from humans produced this P-type 226 activity [65]. Large heat-labile non-bacteriolytic bacteriocin [7,66].

4. Bacteriocins in *Streptococcus pyogenes*

The characterization of bacteriocin activity in *Streptococcus pyogenes*, particularly due to the lantibiotics SA-FF22, streptin, and salivaricin A, has greatly advanced our understanding of their roles in bacterial niche adaptation, host interactions, and microbial antagonism.

4.1. SA-FF22

The discovery and characterization of SA-FF22 marked a pivotal milestone in the study of bacteriocins from Gram-positive bacteria, particularly among *Streptococcus pyogenes* [67–69]. Isolated from a serotype M52 strain, SA-FF22 was the first lantibiotic identified in *S. pyogenes* and classified as a Class I lantibiotic due to its hallmark post-translational modifications, including dehydrated amino acids and thioether cross-links. Structurally,

it shares significant similarity with nisin, the prototypical lantibiotic from *Lactococcus lactis*, suggesting a shared evolutionary lineage for lantibiotics across diverse bacterial genera [7,35]. This discovery challenged early assumptions that bacteriocin production was primarily a feature of commensal or environmental bacteria, demonstrating instead that even pathogenic species can produce broad-spectrum antimicrobial compounds.

Notable aspects of SA-FF22 biology emerged through subsequent studies. The bacteriocin determinants were shown to be transferable between *S. pyogenes* strains via bacteriophage-mediated transduction [70], highlighting a mechanism for horizontal gene transfer. Furthermore, a distinctive inhibition pattern, designated as P-type 436, was identified by application of the bacteriocin fingerprinting scheme, with a notable prevalence of this P-type found in serotype M49 isolates. Interestingly, *Streptococcus dysgalactiae* (e.g., strain 67) produces an identical lantibiotic, and *S. salivarius* strains, such as G32, produce homologous inhibitory substances, collectively known as salivaricin G32 (SalG32) [46]. Strains exhibiting SA-FF22-like activity display cross-immunity to each other's bacteriocins, emphasizing a conserved functional cluster among these bacteria.

SA-FF22 also exhibits several interesting phenotypic traits. It is uniquely produced by cell-wall-deficient L-forms of *S. pyogenes* [71], and its production is upregulated under anaerobic conditions in specific strains, particularly those of serotype M49 [23]. This indicates a potentially specialized role for SA-FF22 in oxygen-limited environments, such as deep tissue infections encountered in invasive streptococcal diseases like necrotizing fasciitis. Moreover, the emergence of bacteriocin-negative variants during growth at elevated temperatures implied that the genetic determinants for SA-FF22 might be plasmid-associated [72].

The discovery of SA-FF22 underscored the multifaceted roles of lantibiotics—not only as antimicrobial agents targeting competing microbes but also as potential modulators of host–pathogen interactions. Its study provided early insights into bacteriocin-mediated microbial ecology, plasmid and phage-associated gene mobility, and the evolutionary convergence of streptococcal lantibiotic production. The continued exploration of SA-FF22 and related lantibiotics remains crucial in understanding the intricate interplay of bacteriocin activity within both commensal and pathogenic streptococcal populations.

4.2. Salivaricin A

Salivaricin A was initially identified as a bacteriostatic inhibitory agent produced by isolates of *S. pyogenes* serotype M4T4, where it was originally referred to as "the inhibitor" [38]. P-typing of these isolates yielded a distinctive P-type 655 profile. Early epidemiological studies—such as those examining long-term oral carriage of *S. pyogenes* in schoolchildren—revealed a predominance of inhibitor-positive M4T4 isolates, underscoring the potential importance of bacteriocin-mediated microbial competition as a key component of their niche adaptation strategy [31]. By inhibiting competing microbes, including commensal streptococci, salivaricin A may facilitate stable colonization of the pharyngeal mucosa. The coexistence of bacteriocin production with alternative virulence mechanisms in M4 strains highlights a multifaceted strategy for persistence in the human host.

Interestingly, the *salA* locus is widely distributed among *S. pyogenes* strains. A variant structural gene, salA1, is present in over 90% of these strains; however, functional SalA1 production is typically restricted to serotype M4T4 strains. Other *S. pyogenes* have deletions within genes required for processing or transport, curtailing production of the active lantibiotic. In this context, it has been proposed that the retention of immunity determinants against salivaricin A offers a significant survival advantage. For *S. pyogenes*, protection against exogenous cationic antimicrobial peptides—potentially from competing microbes or the host immune system—may be more critical than the energetic cost of synthesizing the lantibiotic [73].

4.3. Streptin

Streptin is a type A1 lantibiotic produced by *S. pyogenes* and is associated with distinctive P-type 777 BLIS fingerprint activity (viz., it is strongly inhibitory to all 9 standard indicators). It exhibits a broad inhibitory spectrum, targeting a range of streptococcal species, including *Streptococcus pneumoniae*, *S. salivarius*, *S. uberis*, many strains of *S. mutans*, and most *S. sanguinis*. Additionally, streptin effectively inhibits all tested streptococci from Lancefield Groups A through T, highlighting its considerable antimicrobial potential [18]. Molecular characterization has revealed that streptin exists in two major forms. Streptin 1 is a 23-amino-acid peptide with a molecular mass of 2424 Da, whereas streptin 2 contains three additional amino acids at the N-terminus. Both forms were purified from a serotype M25 *S. pyogenes* using reversed-phase chromatography and were found to induce their own production in cultures, demonstrating an autoregulatory function [18].

The genetic determinants for streptin biosynthesis reside within a dedicated gene cluster. The srtA gene encodes the structural precursor of the lantibiotic and is widely present across *S. pyogenes* strains. However, functional streptin production is observed in only 10 of 40 *srtA*-positive strains. This limited expression is likely due to deletions or deficiencies in key auxiliary genes (*srtB*, *srtC*, *srtT*), which are responsible for processing and transporting the prepeptide, or due to impaired transcriptional activation of *srtA* [18].

Intriguingly, a homologous streptin-like gene has also been identified in some *S. salivarius* strains, often carried on megaplasmids. However, active streptin production is rare in *S. salivarius*. In *S. pyogenes*, the activation of streptin is mediated by a speB-encoded streptococcal proteinase [18]. In contrast, *S. salivarius* may lack an enzyme with the required specificity or may not express the protease and prepeptide under compatible conditions.

The evolving knowledge of streptin—its broad-spectrum activity, molecular diversity, regulatory mechanisms, and interspecies variability—underscores its potential both as a novel antimicrobial agent and as a model for understanding lantibiotic regulation in streptococci. Further investigations into its biosynthetic pathways and regulatory networks, particularly in contrasting *S. pyogenes* and *S. salivarius*, could open new avenues for therapeutic applications in bacterial infection control.

4.4. Streptococcin A-M57

Streptococcin A-M57 (SA-M57) is a novel non-lantibiotic bacteriocin produced by *S. pyogenes* strains of M-type 57. It was first identified by its distinctive inhibition pattern, designated as P-type 614, across all 35 independent M-57 isolates tested using the BLIS fingerprinting assay [21].

SA-M57 production is notably enhanced in the presence of blood and under alkaline conditions. This suggests that environmental factors modulate its synthesis, potentially reflecting its role in natural host niches [21]. The bacteriocin is secreted in two molecular weight forms, estimated by Sephadex G-100 chromatography:

- SA-M57 alpha: >100,000 Da.
- SA-M57 beta: ~33,000 Da.

Despite the difference in size, both forms exhibit identical antimicrobial activity. They are also heat- and protease-sensitive, indicating that their protein structure is crucial for function. Additionally, the bacteriocin's activity is potentiated in the presence of human plasma, suggesting that host-derived factors may strongly influence its inhibitory effects [21].

SA-M57 is encoded by the pDN571 plasmid (3351 bp), belonging to the pC194/pUB110 family of rolling-circle plasmids. This plasmid contains three open reading frames, including scnM57, which encodes SA-M57 as a 179-amino-acid polypeptide with a 27-residue N-terminal secretion signal peptide for extracellular release [20].

Genetic studies have demonstrated that SA-M57 production is independent of M protein expression [20]. The distinct inhibitory profile of SA-M57 (P-type 614) and its environmental regulation suggest a role in bacterial competition within the host. The rolling-circle plasmid nature of pDN571 highlights the potential for horizontal gene transfer among *S. pyogenes* populations, which may contribute to the ecological fitness of M-type 57 strains. The fact that SA-M57 production is independent of M protein expression further suggests that it serves as a separate adaptive trait, possibly aiding survival in competitive microbial environments.

Another study indicated that similar plasmid-encoded antimicrobial activity is also observed in an M-type 69 *S. pyogenes* [37]. This suggests that the production of bacteriocins like SA-M57 may be distributed among different M-types of *S. pyogenes*.

4.5. Distribution of Currently Characterized and Putative BLIS Activities in S. pyogenes

Preliminary bacteriocin fingerprinting combined with PCR probing investigations of a large collection of *S. pyogenes* strains, representing over 100 M-serotypes (or emm types), provides a comprehensive overview of the diversity and distribution of known and putative novel bacteriocin loci (Table 3). The table summarizes P-type patterns, associated emm/M-types, identified bacteriocins, and bacteriocin genetic loci (structural and immunity genes), highlighting the breadth of the distribution of bacteriocin loci and raising important questions regarding their implications for microbial interactions and pathogenicity. Table 3 shows that there can be divergence between the P-type score and what is present in the genome based on PCR. The rationale for this divergence is the non-expression of the respective lantibiotic in the conditions of a P-type. The non-expression is likely driven by the significant metabolic cost to the cell. Clearly, there is selective pressure to obtain the genetic basis for lantibiotic production and immunity due to the presence of lantibiotics in *S. pyogenes'* environmental niche. However, given the significant costs of production, the synthesis of lantibiotics is either lost or heavily repressed while immunity is maintained to optimize survival in the niche.

Table 3. PCR detection of salivaricin A, streptin, and SA-FF22 structural and immunity coding genes in *S. pyogenes* by Tagg et al. [74].

Emm/M Type	P-Type	Salivaricin A	Streptin	SA-FF22	Emm/M Type	P-Type	Salivaricin A	Streptin	SA-FF22
M1	004	Yes	Yes	No	M67	004	Yes	Yes	No
M2	204	Yes	Yes	No	M68	324	Yes	Yes	No
M3	324	Yes	Yes	No	M69	324	Yes	No	No
M4	657	Yes +	Yes	No	M70	326	Yes	No	No
M5	000	Yes	No	No	M71	777	Yes	Yes +	No
M6	000	Yes	No	No	M72	000	Yes	No	No
M8	324	Yes	Yes	No	M73	324	No	Yes	No
M9	324	Yes	Yes	No	M74	226	Yes	No	No
M11	774	No	Yes +	No	M75	204	Yes	Yes	No
M12	774	Yes	Yes +	No	M76	777	Yes	Yes +	No
M13	004	Yes	Yes	No	M77	324	Yes	No	Yes
M14	204	Yes	No	No	M78	004	Yes	No	No
M15	234	Yes	Yes	No	M79	324	Yes	Yes	No
M17	000	Yes	No	No	M80	004	Yes	No	No
M18	000	Yes	No	No	M81	000	IM	Yes	No
M19	000	Yes	Yes	No	emm82	324	Yes	Yes	No
M22	000	Yes	No	No	emm83	577	Yes	No	Yes +

Table 3. *Cont.*

Emm/M Type	P-Type	Salivaricin A	Streptin	SA-FF22	Emm/M Type	P-Type	Salivaricin A	Streptin	SA-FF22
M23	004	Yes	Yes	No	emm84	674	Yes	Yes	No
M24	000	Yes	Yes	No	emm85	204	Yes	Yes	No
M25	774	Yes	Yes +	No	emm86	657	Yes +	No	No
M26	004	Yes	Yes	No	emm87	000	Yes	Yes	No
M27	774	Yes	Yes +	No	emm88	000	Yes	No	Yes +
M28	776	Yes	Yes	No	emm89	004	Yes	Yes	No
M29	004	Yes	Yes	No	emm90	000	Yes	Yes	No
M30	000	Yes	Yes	No	emm91	000	Yes	No	No
M31	324	Yes	Yes	No	emm92	000	Yes	Yes	No
M32	000	Yes	No	No	emm93	000	No	No	No
M33	324	Yes	No	No	emm94	324	Yes	Yes	No
M34	000	Yes	Yes	No	emm95	000	Yes	No	No
M36	004	Yes	No	No	emm96	304	Yes	Yes	No
M37	000	No	No	No	emm97	777	Yes	No	Yes +
M38	010	Yes	No	No	emm98	324	Yes	No	No
M39	000	Yes	Yes	No	emm99	654	Yes +	No	No
M40	000	Yes	No	No	emm100	454	Yes	No	No
M41	004	Yes	No	No	emm101	004	Yes	No	No
M42	004	Yes	Yes	No	emm102	004	Yes	Yes	Yes
M43	000	Yes	No	No	emm103	000	Yes	Yes	No
M44	204	Yes	Yes	No	emm104	000	Yes	No	No
M46	-	Yes	Yes	No	emm105	000	Yes	Yes	Yes
M47	204	Yes	No	No	emm106	000	No	Yes	No
M48	324	IM	Yes	No	emm107	224	Yes	Yes	No
M49	324	IM	Yes	No	emm108	204	Yes	No	No
M50	000	Yes	Yes	No	emm109	324	Yes	Yes	Yes
M51	004	Yes	No	No	emm110	726	Yes	No	No
M52	000	Yes	No	No	emm111	726	Yes	No	No
M53	004	Yes	No	No	emm112	726	Yes	No	No
M54	004	Yes	Yes	No	emm113	577	Yes	Yes	Yes +
M55	004	Yes	Yes	No	emm114	226	Yes	Yes	No
M56	004	Yes	No	No	emm115	204	Yes	No	No
M57	614	Yes	Yes	Yes	emm116	204	No	No	No
M58	324	No	Yes	Yes	emm117	000	Yes	No	No
M59	324	Yes	No	No	emm118	226	Yes	Yes	No
M60	777	Yes	Yes +	No	emm119	000	Yes	No	No
M61	234	Yes	Yes	No	emm120	204	Yes	No	No
M62	324	Yes	Yes	No	emm121	004	Yes	No	No
M63	324	Yes	Yes	No	emm122	626	Yes	Yes	No
M64	000	Yes	Yes	No	emm123	600	Yes	Yes	No
M65	324	Yes	No	No	emm124	204	No	Yes	No
M66	774	Yes	Yes +	No					

+ = has antimicrobial profile consistent with the expression of this lantibiotic; IM = immunity only.

The currently uncharacterized P-type patterns suggest the presence of additional, unidentified *S. pyogenes* bacteriocins. Genomic and proteomic analyses of strains exhibiting unique P-type patterns are expected to unveil additional antimicrobial determinants, fur-

ther expanding the repertoire of bacteriocins associated with *S. pyogenes*. These findings highlight the untapped diversity of bacteriocins within *S. pyogenes* and reinforce the idea that even well-studied pathogens may harbor undiscovered antimicrobial systems [75]. Elucidating the structure, function, and ecological role of these putative bacteriocins will likely provide further insights into the passive and aggressive interactions of *S. pyogenes* within its human host and could open new possibilities for therapeutic interventions.

4.6. Broader Significance of Bacteriocin Loci in S. pyogenes

The near-universal presence of bacteriocin immunity loci in *S. pyogenes*—even in strains that lack active bacteriocin production—underscores their essential role in adaptation. These loci not only protect the bacterium against their own bacteriocins and homologous exogenous bacteriocins produced by competitors but also confer resistance to host-derived cationic antimicrobial peptides (e.g., defensins), thereby mitigating innate immune pressures and enhancing survival within polymicrobial environments [73].

Phenotypic approaches, such as bacteriocin fingerprinting, have uncovered the hidden diversity of bacteriocins in *S. pyogenes*. Many of these bacteriocins belong to the lantibiotic class. Lantibiotic regulatory systems are typically encoded in conserved, multi-operonic gene clusters that include not only the pre-peptide and modification enzymes but also dedicated secretion and immunity proteins. These clusters are frequently linked to two-component signal transduction systems that detect the mature lantibiotic and trigger autoinduction, enabling cell-density-dependent regulation of bacteriocin production [76].

In *S. pyogenes*, homologous systems similar to the *salivaricin A* locus found in *S. salivarius* have been identified. Although many *S. pyogenes* strains harbor mutations or deletions in biosynthetic genes—potentially precluding the generation of an active lantibiotic—the preservation of immunity and regulatory elements (e.g., sensor kinases and response regulators) implies that these loci still fulfill critical roles. For instance, studies have shown that mutations in regulatory components of the sal cluster (such as *salY* and *salK*) lead to attenuated virulence in infection models, suggesting that even in the absence of active antimicrobial production, these systems contribute to host colonization and survival [73,77].

Moreover, recent studies have underscored the importance of the *sal* lantibiotic locus in the virulence of *S. pyogenes*. Transcriptional analysis of this locus reveals complex regulation, where the internal *salKR* promoter, regulated by the *SalR* response regulator, plays a key role in modulating expression of genes critical for virulence [76]. These regulatory mechanisms likely contribute to *S. pyogenes*' ability to adapt to fluctuating host environments and evade immune responses.

Interestingly, the *SalA* lantibiotic locus is also involved in interspecies signaling. Studies have demonstrated that the *SalA* peptide from *S. salivarius* can inhibit the growth of *S. pyogenes*, with the activity of *SalA* being autoregulated and potentially influencing bacterial competition within the oral microbiota [77,78]. These findings highlight the potential ecological role of lantibiotics in shaping microbial community dynamics and their implications for host–microbe interactions.

In the context of increasing antibiotic resistance, the dual functionality of these bacteriocin systems—combining direct antimicrobial activity with sophisticated regulatory networks—may offer novel avenues for therapeutic intervention. The use of probiotic *S. salivarius* strains producing lantibiotics, such as *SalA*, demonstrates promising potential to inhibit *S. pyogenes* colonization and mitigate infections, supporting the concept that bacteriocin-mediated modulation of microbial populations could play a role in novel treatment strategies [6,79].

Additionally, studies have indicated that bacteriocin-like peptides such as *SalA* might be key in enhancing protection against *S. pyogenes* by promoting their sustained presence

in the host. For example, the ingestion of *SalA*-producing *S. salivarius* strains resulted in increased inhibitory activity against *S. pyogenes* in human saliva, pointing to a possible role for these probiotics in controlling *S. pyogenes* infections in the oral cavity [79].

As *S. pyogenes* continues to challenge current therapeutic approaches, understanding and harnessing the bacteriocin systems within this pathogen and its interactions with other microbial species will be critical for developing innovative strategies to combat infections and manage antibiotic resistance [80].

5. Bacteriocins in *Streptococcus salivarius*

The bacteriocins produced by *Streptococcus salivarius* highlight the remarkable ability of this benign commensal to thrive within the complex and competitive microbiota of the oral cavity. Through targeted microbial inhibition, these antimicrobial peptides enable *S. salivarius* to maintain its ecological dominance while also contributing to human health.

5.1. Salivaricin A and Its Variants

Salivaricin A (SalA) is a lantibiotic, first identified in serotype M4 *Streptococcus pyogenes* and proposed (see above) to potentially be a factor in establishing its niche in a long-term carriage state within the human oral microbiota [31]. Subsequently, SalA and its variants have been found also to be widely distributed in *S. salivarius*. The SalA-producing commensal streptococci demonstrate bacteriostatic inhibitory activity against any *Streptococcus pyogenes* that are expressing the SalA immunity locus [78]. The production of SalA highlights two key ecological roles of *S. salivarius*:

1. Ecological Competence—SalA enhances the ability of *S. salivarius* to colonize the oral cavity by suppressing the proliferation of competing microbial species.
2. Commensal Harmony—despite its antimicrobial properties, SalA-producing *S. salivarius* strains coexist harmlessly with their human hosts, thereby supporting their probiotic potential [32,81,82].

SalA is classified as a type AII lantibiotic and shares structural and functional similarities with nisin, albeit with a narrower activity spectrum focused on oral and upper respiratory tract bacteria. The *sal* gene cluster, responsible for SalA biosynthesis, consists of eight genes encoding structural (*salA*), modification (*salB*, *salC*), transport (*salT*), immunity (*salX*, *salY*), and regulatory (*salR*, *salK*) functions [83]. Autoregulation of SalA production is mediated by the two-component SalKR system, which modulates gene expression in response to external SalA concentrations. In *S. salivarius* 20P3, inactivation of *salB* abolished SalA production and immunity, confirming the essential role of these genes in lantibiotic biosynthesis and self-protection [78].

Collectively, these findings illustrate that salivaricin A functions not only as an antimicrobial agent but also as an intra- and interspecies signaling molecule. In *S. salivarius*, active production of SalA contributes directly to microbial competition in the oral cavity. In contrast, for *S. pyogenes*, the strategic retention of SalA immunity may be the preferred survival trait, allowing these pathogens to mitigate the effects of host-derived and microbial cationic peptides without the metabolic burden of producing the lantibiotic itself. This dual role of salivaricin A and its variants highlights its ecological and evolutionary significance within the complex oral microbiome.

Over time, multiple structural variants of SalA have been identified, reflecting adaptation to evolutionary pressures within the oral microbiota. Wescombe et al. [77] described four additional variants (SalA2 to SalA5), each exhibiting antimicrobial activity against *Micrococcus luteus* and acting as inducers of SalA production. SalA2, isolated from *S. salivarius* K12, was characterized structurally and functionally, revealing that its N-terminal region is crucial for lipid II binding and antibacterial activity [84]. Truncation of the first

two residues abolished bioactivity, confirming the importance of the positively charged N-terminal amino acids.

As a predominant colonizer of the human tongue and buccal mucosa, *S. salivarius* plays a key ecological role in maintaining oral microbial balance. SalA production enhances this role through the following:

- Competitive Advantage—By inhibiting *S. pyogenes*, SalA-producing *S. salivarius* strains promote their own persistence and limit pathogen colonization. Despite their antimicrobial properties, SalA-producing *S. salivarius* strains coexist harmlessly with their human hosts, supporting their probiotic potential.
- Oral Health Applications—strains such as BLIS K12, which produce SalA, have demonstrated clinical efficacy in reducing streptococcal pharyngitis and modulating the oral microbiome in favor of commensal populations [32,77,81,82].

Salivaricin A represents a key antimicrobial factor contributing to the ecological success of *S. salivarius*. Given the growing interest in probiotic applications, SalA-producing *S. salivarius* continue to provide an effective contribution as natural biotherapeutics for oral and upper respiratory tract health.

5.2. Salivaricin B

Salivaricin B is a 25-amino acid polycyclic peptide belonging to the type AII lantibiotics. Distinct from salivaricin A, salivaricin B exhibits a broader inhibitory spectrum—targeting both Gram-positive and some Gram-negative bacteria—which broadens the antimicrobial arsenal of *S. salivarius* expressing the functional locus. Originally identified as a lantibiotic apparently unique to *S. salivarius*, salivaricin B represents a significant adaptive mechanism in polymicrobial environments like the human oral cavity. Its production in the probiotic strain BLIS K12 is particularly notable because salivaricin B is encoded on the 190-kb transmissible megaplasmid pSsal-K12 alongside salivaricin A2 [12]. This co-localization underscores the evolutionary advantage provided by the simultaneous expression of multiple bacteriocins, facilitating competitive exclusion of pathogenic bacteria while preserving the integrity of the commensal microbiota. Early work by Tagg and Russell [50] anticipated the potential of such inhibitor-producing strains for controlling dental caries and upper respiratory tract infections.

New insights into the mode of action of salivaricin B have been provided by Barbour et al. [85]. Their study revealed several key aspects of its bactericidal activity against Gram-positive bacteria: It was found that salivaricin B requires micro-molar concentrations to exert its bactericidal effect, in contrast to the nano-molar potency of the prototype lantibiotic nisin A. Also, unlike nisin A, salivaricin B does not induce pore formation or dissipate the membrane potential in target cells. Instead of disrupting the membrane directly, salivaricin B was shown to interfere with cell wall synthesis, as evidenced by the accumulation of the soluble cell-wall precursor UDP-MurNAc-pentapeptide—a key building block of peptidoglycan [85]. Transmission electron microscopy of treated cells further confirmed a reduction in cell wall thickness and aberrant septum formation, while the cytoplasmic membrane remained intact.

Salivaricin B contributes significantly to the ecological fitness of *S. salivarius* within the oral microbiome. Its broad-spectrum activity allows salivaricin B-producing strains to inhibit a wide range of oral pathogens. In communities with lower microbial diversity, these strains can dominate and maintain a competitive edge, thereby preserving the stability of the oral ecosystem. Furthermore, the localization of the salivaricin B gene cluster on a transmissible megaplasmid facilitates horizontal gene transfer, potentially aiding the dissemination of bacteriocin-mediated competitive traits among *S. salivarius* populations [12].

The unique mode of action and broad-spectrum inhibitory capacity of salivaricin B highlight its potential as a novel biotherapeutic agent in the prevention and treatment of oral and upper respiratory tract infections.

5.3. Salivaricin 9

Salivaricin 9 (Sal9) is a relatively recently characterized Type A2 lantibiotic produced by *S. salivarius*. With a molecular weight of 2560 Da and sharing only 46% amino acid identity with its closest known homolog (SA-FF22), Sal9 is structurally distinct within the salivaricin family [48].

The genetic locus responsible for Sal9 production, designated as *siv*, was first identified in *S. salivarius* strain 9 and mapped to an approximately 170 kb megaplasmid that also harbors the gene cluster for salivaricin A4. Interestingly, in strain JIM8780, the *siv* locus was found to be integrated into the chromosome—the first documented instance of a chromosomally encoded lantibiotic locus in *S. salivarius* [48]. Sal9 production is autoinducible; extracts containing Sal9 stimulate its own biosynthesis in both plasmid-associated and chromosomal contexts [49].

Salivaricin 9 exerts its antimicrobial effect by targeting the cytoplasmic membrane of susceptible bacteria. Membrane permeabilization assays and scanning electron microscopy have demonstrated that Sal9 induces pore formation, leading to rapid cell death [86]. Sal9 is notably stable, retaining activity over a broad pH range (2–10) and at high temperatures (90–100 °C). However, its activity is lost upon treatment with proteolytic enzymes such as proteinase K, underscoring its proteinaceous nature [86].

Screening studies have shown that the *sivA* gene is unique to *S. salivarius*, being present in nearly half of the tested strains. Sal9 displays a broad inhibitory spectrum that includes *S. pyogenes*, *Lactococcus lactis*, *Enterococcus hirae*, *Staphylococcus aureus*, and *Streptococcus uberis*, suggesting it plays a significant role in shaping the oral microbial ecosystem [48]. In probiotic applications, strains such as *S. salivarius* M18 produce Sal9 alongside other lantibiotics (SalA and salivaricin M), thereby offering a multifaceted antimicrobial defense that minimizes the risk of resistance development in target pathogens [86].

5.4. Salivaricin G32

Salivaricin G32 is a lantibiotic produced by *S. salivarius* strain G32 and belongs to the SA-FF22 cluster of nisin-like lantibiotics. With a molecular weight of 2667 Da, it is structurally similar to the *S. pyogenes* lantibiotic SA-FF22. Notably, the primary structural difference between salivaricin G32 and SA-FF22 is the absence of a lysine residue at position 2 in salivaricin G32, a feature that contributes to its distinctive antimicrobial profile [46].

The gene encoding salivaricin G32 (*slnA*) is detected in approximately 23% of BLIS-producing *S. salivarius*. In several strains, the *slnA* locus is located on megaplasmids, facilitating horizontal gene transfer among commensal populations [46].

Salivaricin G32 exhibits potent antimicrobial activity against a range of streptococcal species, including *S. pyogenes*. However, strains of *S. pyogenes* that produce SA-FF22 are resistant to salivaricin G32, indicating that the immunity mechanisms associated with SA-FF22 confer cross-protection. Production of salivaricin G32 is inducible; extracts from strains that produce either salivaricin G32 or SA-FF22 can mutually induce the synthesis of the respective lantibiotic, although the precise inducing molecule has yet to be conclusively identified [46].

In the oral microbiome, the production of salivaricin G32 by commensal *S. salivarius* strains likely contributes to the suppression of pathogenic bacteria such as *S. pyogenes*. This targeted antimicrobial activity helps maintain a balanced microbial ecosystem and supports

oral health. Additionally, salivaricin G32 has been identified in other streptococcal species, such as *Streptococcus dysgalactiae*, suggesting a broader distribution within the genus [46].

5.5. Salivaricin E

Salivaricin E is a 32-amino acid, 3565.9 Da lantibiotic produced by the oral probiotic candidate *S. salivarius* strain JH. Notably, it exhibits potent inhibitory activity against *Streptococcus mutans*, a primary bacterium implicated in dental caries. This property positions salivaricin E as an attractive antimicrobial agent for oral health applications [47].

Strain JH is also notable in that it produces high levels of dextranase, an enzyme that degrades dextran—a major component of the exopolysaccharide (EPS) matrix in dental plaque. The combined action of salivaricin E and dextranase not only reduces pathogen adherence but may also enhance the efficacy of other antimicrobials, such as zoocin A, against *S. mutans* embedded within biofilms [47].

The biosynthesis of salivaricin E is encoded by a 14-gene locus (including the key structural gene, srnA) located on a 220 kb megaplasmid (pSsal-JH) in strain JH. The biosynthesis of salivaricin E relies on a single modifying enzyme (*srnM*) and an associated ABC transporter (*srnT*), which likely contributes to its secretion and self-immunity. Its primary structure is unique, sharing only moderate identity (50–53%) with other salivaricins and underscoring its novelty [47]. The salivaricin E locus comprises genes involved in modification, regulation, immunity, and transport, suggesting a coordinated mechanism for efficient lantibiotic production. The genetic organization hints at potential acquisition via transposition and integration with other antimicrobial systems within the strain, ensuring a multifaceted approach to controlling oral pathogens.

It has been speculated that by combining the direct inhibition of *S. mutans* with enzymatic degradation of its biofilms—and working synergistically with other bacteriocins—salivaricin E exemplifies a multi-pronged strategy that underpins the anti-cariogenic potential of *S. salivarius* strain JH. These properties make salivaricin E a promising candidate for future dental therapeutics and probiotic applications aimed at improving oral health.

5.6. Salivaricin M

Salivaricin M (SalM) is a 32-amino acid lantibiotic produced by *S. salivarius* strain M18—a probiotic marketed by BLIS Technologies Ltd. for tooth and gum health. With an approximate mass of 3640 Da, SalM is post-translationally modified with lanthionine rings, as evidenced by blank residues during N-terminal amino acid sequencing, despite its propeptide showing no homology to other lantibiotics. Genomic analysis indicates that the putative genetic locus for SalM is chromosomally encoded; however, when strain M18 is cured of its large megaplasmid—which houses the biosynthetic loci for salivaricins A and 9—SalM activity is lost. This suggests that the production of SalM may rely on shared modification enzymes or an unknown regulatory factor encoded on the megaplasmid [49].

Salivaricin M displays potent inhibitory activity against *S. mutans*, a key bacterium implicated in dental caries, underscoring its clinical relevance in oral health. The unique antimicrobial activity of SalM, combined with the probiotic properties of strain M18, makes it a promising candidate for the development of oral health products aimed at preventing dental caries. Future research to fully elucidate the genes required for its biosynthesis and the regulatory mechanisms governing its production could pave the way for enhanced production and more effective clinical applications.

5.7. P-Type 226 Bacteriocins

The P-type 226 bacteriocins in *S. salivarius* represent a unique group of heat-labile, non-lantibiotic peptides that specifically inhibit *S. pyogenes* when evaluated using the bacteriocin fingerprinting scheme [3]. A large-scale study in Dunedin, New Zealand, found

that approximately 11% of schoolchildren harbored *S. salivarius* strains exhibiting this P-type 226 inhibitory activity, although the presence of these strains was not associated with a reduced incidence of *S. pyogenes* colonization—possibly due to the lack of expression of the inhibitory activity in saliva [87].

A representative P-type 226 strain, designated SN, was further characterized to produce the bacteriocin salivaricin SN, a muralytic enzyme that targets the bacterial cell wall. Salivaricin SN inhibits 18 out of 20 tested *S. pyogenes* strains while showing no activity against other streptococci, and it is inactivated after 30 min at 80 °C, indicating its heat-sensitive nature. Its N-terminal sequence (NH_2-DINGGANTPGAYD-COOH) has been determined, though the full biosynthetic locus remains to be elucidated [14]. Similar non-lantibiotic bacteriocins have been identified in other streptococcal species, such as zoocin A from *S. equi* subsp. *zooepidemicus* [59] and stellalysin from *Streptococcus constellatus* [19], supporting the concept that these narrowly targeted bacteriocins may have promising applications as topical or targeted therapies to control *S. pyogenes* infections without disturbing the broader commensal oral microbiota.

5.8. Sal MPS

Salivaricin MPS is a non-lantibiotic bacteriocin produced by *S. salivarius* strain MPS (P-type 626) that exhibits potent, specific activity against *S. pyogenes*. Its production is constitutive, with activity detectable in liquid cultures within 4–5 h, and is enhanced by the presence of saliva and under anaerobic conditions. Notably, salivaricin MPS effectively kills actively growing *S. pyogenes*, making it a promising candidate for targeted antimicrobial applications. Biochemical analysis has revealed that the purified protein has an approximate molecular mass of 63 kDa, and N-terminal sequencing (NH_2-DEQAAVSDSTTSITSDNGVV) has confirmed its identity. The structural gene, salMPS, has been identified, with two variants differing primarily in their central region (amino acids 210–345) while retaining nearly identical N- and C-terminal segments, and both encode proteins similar to cell-wall-remodeling enzymes found in other streptococci (unpublished) [49,88].

In the probiotic strain *S. salivarius* BLIS M18, additional genetic factors, possibly linked to a megaplasmid that also carries loci for other bacteriocins, appear necessary for full expression. Furthermore, in some strains (e.g., MPS), the production of other bacteriocins such as SalA is compromised (e.g., due to a stop codon mutation), thereby emphasizing the unique contribution of salivaricin MPS to the anti-*S. pyogenes* profile. Together, these findings underscore the potential of salivaricin MPS as a targeted antimicrobial agent for oral health, warranting further research into its genetic regulation and clinical applications [44,49].

5.9. Unresolved P-Type Patterns

Bacteriocin fingerprinting has revealed numerous uncharacterized P-type patterns in *S. salivarius*, hinting at the existence of novel lantibiotics or other antimicrobial peptides. These patterns are often linked to strains found in unique ecological niches, indicating potential adaptations to specific environments. Ongoing studies aim to uncover the genetic loci associated with these patterns and characterize the peptides they encode. Characterizing these novel bacteriocins presents an exciting opportunity to expand the understanding of microbial competition and the ecological dynamics within the oral cavity. Some of these peptides may have applications beyond oral health, potentially benefiting skin, respiratory, and other body-site microbiomes.

5.10. Future Prospects in Therapeutic Applications

The ongoing exploration of *S. salivarius* lantibiotics and other bacteriocin activities, including those initially detected through their production of novel P-type patterns, holds

considerable promise for developing novel therapeutic approaches. By investigating these uncharacterized BLIS activities, researchers may uncover new antimicrobial compounds with applications in treating both oral and systemic infections. Further studies will enhance the understanding of *S. salivarius*'s role as a probiotic and its potential to shape the microbial ecosystems of various body sites.

6. The Broader Significance of Streptococcal Bacteriocins

6.1. For the Bacteria

Ecological Roles

The ubiquity of bacteriocins indicates they are crucial tools for microbial competition, colonization, and survival, playing a key role in the ecological fitness of bacteria. These molecules act both as antimicrobial agents and signaling peptides, conferring distinct advantages in densely populated environments. Bacteriocins facilitate microbial competition by selectively targeting susceptible competitors while sparing those with immunity mechanisms. For example, *S. pyogenes* uses SA-FF22 and other bacteriocins to establish a presence in polymicrobial environments such as the oropharynx, while *S. salivarius* produces salivaricins to suppress the excessive proliferation of pathogens like *S. pyogenes* in the oral cavity. The competitive edge associated with bacteriocin expression often results in enhanced colonization potential, as seen with salivaricins aiding in *S. salivarius* colonization of the tongue and buccal mucosa. Additionally, immunity loci linked to bacteriocin production allow bacteria to evade self-destruction and resist host-derived antimicrobial peptides. For *S. pyogenes*, salivaricin A immunity loci can provide cross-protection against cationic antimicrobial peptides produced by the human host. These multifaceted roles of bacteriocins underscore their importance in both pathogenic and commensal lifestyles. Both *S. pyogenes* and *S. salivarius* leverage bacteriocins for ecological fitness, but their strategies diverge based on their distinct roles within the human host:

- Pathogen (*S. pyogenes*): Lantibiotics like SA-FF22 enhance *S. pyogenes*' ability to gain entry and then dominate competitive niches. Immunity loci further assist in evading innate host defenses, strengthening its capacity to increase numbers.
- Commensal (*S. salivarius*): in contrast, *S. salivarius* uses bacteriocins primarily to protect its niche, promoting its role as a beneficial oral commensal.

6.2. For the Human Host

6.2.1. Pathogenic Potential in *S. pyogenes*

The immunity loci associated with lantibiotics in *S. pyogenes* play a dual role by enabling the bacterium to thrive in the host environment while resisting innate immune responses. Understanding these loci could reveal molecular mechanisms underlying *S. pyogenes* pathogenicity and identify potential therapeutic targets.

6.2.2. Natural Defense by *S. salivarius*

On the other hand, *S. salivarius*'s production of lantibiotics like salivaricin A enhances the host's natural defense mechanisms, reducing the incidence of infections in the oral cavity. Strains of *S. salivarius* that produce salivaricins have shown promise in modulating the oral microbiota and preventing pharyngeal infections, positioning them as effective probiotics.

6.2.3. Translational Potential in Therapeutics

The study of bacteriocins presents significant opportunities for developing novel antimicrobial and probiotic therapies. Lantibiotics' specificity and potency make them strong candidates as novel antibiotics, particularly for combating resistant pathogens [30]. Moreover, harnessing lantibiotic-producing commensals like *S. salivarius* can help initiate

probiotic interventions such as BLIS K12 and BLIS M18, offering sustainable alternatives to conventional antibiotics while preserving microbiota diversity.

7. Future Directions

7.1. Unresolved Questions

1. Functions of Newly Detected P-Type Patterns

The discovery of novel P-type patterns presents intriguing questions about their biological roles. Do these patterns correspond to uncharacterized bacteriocins, or might they represent expression of other forms of antimicrobial or signaling peptides? A deeper molecular characterization of these patterns can help uncover new aspects of microbial communication, competition, and host–microbe interactions, expanding our understanding of microbial ecology.

2. Persistence of Immunity Loci Without Active Bacteriocin Production in *S. pyogenes*

The frequent presence and expression of immunity loci in *S. pyogenes* strains that do not actively produce lantibiotics suggests that this confers an evolutionary advantage. These loci may serve either as an energy-efficient specific defense against lantibiotics produced by other microbes and cationic effector molecules associated with the immune system of the human host. Alternatively, they may be remnants of an ancestral ability to produce functional bacteriocins. Investigating the persistence of these loci could shed light on selective pressures within microbial ecosystems and offer a deeper understanding of bacterial genome evolution.

7.2. Research Opportunities

1. Advancing Discovery Through Genomics, Proteomics, and Machine Learning

Integrating advanced genomic and proteomic technologies has the potential to revolutionize bacteriocin research. Whole-genome sequencing (WGS) can rapidly identify lantibiotic biosynthetic gene clusters, often supported by specialized tools like BAGEL4 and antiSMASH [33,89]. Proteomic approaches confirm their expression and activity. Additionally, techniques such as CRISPR-Cas9 gene editing enable functional validation of gene cluster components, while transcriptomic profiling (e.g., RNA-Seq) can elucidate regulatory networks that control lantibiotic production under different environmental conditions, providing a deeper understanding of their ecological roles and therapeutic potential.

Machine learning is emerging as a powerful tool in antimicrobial peptide (AMP) discovery, and similar approaches could be applied to lantibiotic research. Computational models, such as those used to predict AMPs from genomic data, could assist in identifying novel lantibiotic candidates, predicting their functional properties, and optimizing their therapeutic applications. Multi-label prediction models, such as iAMP-2L and MLAMP [90], have successfully classified AMPs with antibacterial, antifungal, antibiofilm, and anti-inflammatory activities [91]. Applying such predictive frameworks to lantibiotics may reveal previously uncharacterized bioactivities, predict structure–activity relationships, and facilitate rational peptide engineering [92]. By integrating machine-learning-driven predictions with genomic and proteomic analyses, researchers can streamline the discovery pipeline, efficiently prioritizing candidate bacteriocins for experimental validation and accelerating their translation into clinical and biotechnological applications.

2. Translating Lantibiotic Knowledge into Therapeutics

The remarkable specificity and potency of lantibiotics position them as attractive candidates for the development of next-generation therapeutics, especially against multidrug-resistant pathogens. As ribosomally synthesized antimicrobial peptides with

post-translational modifications that confer enhanced stability and activity, lantibiotics offer several advantages over traditional antibiotics [93]. However, translating their promise into clinical applications requires overcoming several hurdles, including susceptibility to proteolytic degradation, limited bioavailability, and narrow pharmacokinetic profiles. To address these challenges, researchers are employing an expanding array of innovative techniques. Synthetic biology is enabling the rational redesign of lantibiotic biosynthetic pathways for improved yield and modifiability, while combinatorial biosynthesis allows the generation of novel analogues with tailored activity spectra. Structure-guided peptide engineering and site-directed mutagenesis are now being used to enhance target specificity, improve resistance to host proteases, and reduce toxicity [94]. Additionally, advanced delivery systems—such as biodegradable nanoparticles, hydrogels, and lipid-based carriers—are also being explored to protect lantibiotics during transit and to facilitate their localized or sustained release at infection sites [95].

Despite these advances, several limitations continue to hamper the clinical adoption of lantibiotics and other bacteriocins [96,97]. Their production often remains costly and complex, particularly when scaled for pharmaceutical use [93]. Furthermore, while resistance development to lantibiotics appears relatively rare, concerns persist about their long-term evolutionary impact on microbial communities, especially with repeated or widespread use. Regulatory and commercial hurdles also present significant obstacles. The classification of bacteriocins—as either biologics, drugs, or food additives—varies across jurisdictions, creating uncertainty in development pipelines. Additionally, the lack of standardized evaluation frameworks for peptide therapeutics complicates the path from discovery to approval [98].

Nevertheless, alongside these direct therapeutic applications, the potential of bacteriocin-producing probiotics—such as *Streptococcus salivarius* strains—to confer ecological advantages in situ is gaining traction. These strategies offer a promising means of re-establishing microbial balance, preventing pathogen colonization, and reducing selective pressures that drive antibiotic resistance—all while leveraging the host's natural microbiota [99]. To realize the full potential of bacteriocins as therapeutics, an integrated approach is required—one that combines the tools of molecular engineering, systems biology, and translational science with robust investment in scalable manufacturing and regulatory harmonization [96,97].

8. Conclusions

The progression from early bacteriocin fingerprinting methodologies to today's sophisticated genomic and proteomic tools has transformed our understanding of bacterial antimicrobial strategies. Early work, such as that by Tagg and Bannister [3], laid the foundation for categorizing bacteriocins based on their inhibitory spectra, while contemporary studies have elucidated the complex genetic regulation and diverse biochemical modifications that underpin these molecules. In particular, the study of lantibiotics and non-lantibiotic bacteriocins in both pathogenic and commensal streptococci has revealed their dual role as microbial weapons and mediators of host–microbe interactions.

In *S. pyogenes*, variable distribution of lantibiotic loci provides insights into the pathogen's strategies for both interbacterial competition and evasion of host immune defenses. The presence of immunity determinants, which may confer cross-protection against host cationic antimicrobial peptides, further highlights their role in promoting bacterial survival and virulence.

In commensal organisms such as *S. salivarius*, bacteriocins contribute to maintaining microbial homeostasis in the oral cavity. Strains producing diverse molecules—including

salivaricins A, B, 9, and M, and non-lantibiotic agents like salivaricin MPS—demonstrate a multifaceted approach to inhibiting key pathogens such as *S. pyogenes* and *S. mutans*.

The bacteriocins of *S. pyogenes* and *S. salivarius* exhibit intriguing similarities in their structure, spectrum of activity, and regulation. These bacteriocins evidently play a significant role in the successful colonization and persistence of their host streptococci within the human host. Additionally, they highlight the potential of utilizing natural microbial defenses for therapeutic purposes. By bridging historical insights with modern innovations, continued exploration of streptococcal bacteriocins promises to reveal new strategies for combating infectious diseases and promoting microbial balance in the human host.

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