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MINIREVIEW

Therapeutic Application of Lantibiotics and Other Lanthipeptides: Old and New Findings

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ABSTRACT Lanthipeptides are ribosomally synthesized and posttranslationally modified peptides, with modifications that are incorporated during biosynthesis by dedicated enzymes. Various modifications of the peptides are possible, resulting in a highly diverse group of bioactive peptides that offer a potential reservoir for use in the fight against a plethora of diseases. Their activities range from the antimicrobial properties of lantibiotics, especially against antibiotic-resistant strains, to antiviral activity, immunomodulatory properties, antiallodynic effects, and the potential to alleviate cystic fibrosis symptoms. Lanthipeptide biosynthetic genes are widespread within bacterial genomes, providing a substantial repository for novel bioactive peptides. Using genome mining tools, novel bioactive lanthipeptides can be identified, and coupled with rapid screening and heterologous expression technologies, the lanthipeptide drug discovery pipeline can be significantly sped up. Lanthipeptides represent a group of bioactive peptides that hold great potential as biotherapeutics, especially at a time when novel and more effective therapies are required. With this review, we provide insight into the latest developments made toward the therapeutic applications and production of lanthipeptides, specifically looking at heterologous expression systems.

KEYWORDS antimicrobial agents, antimicrobial peptides, antiviral agents, drug resistance, heterologous expression systems, lantibiotics, lanthipeptides

Lantibiotics, first described in 1988, are ribosomally synthesized and posttranslationally modified peptides (RiPPs) with antimicrobial activity that contain *meso*-lanthionine and 3-*methyl*-lanthionine (1, 2). While most lanthionine-containing RiPPs are lantibiotics, there are some that lack antimicrobial activity. To account for the few lanthionine-containing RiPPs without antimicrobial activity the overall term "lanthipeptides" is used (3). Modification occurs at the precursor peptide, which consists of an N-terminal leader peptide, important for recognition by modification enzymes, and a C-terminal-modified core peptide (1, 2, 4, 5) (Fig. 1; see Fig. S1A, S2A, S3, and S4 in the supplemental material). Lanthionine cross-links form as result of a sequence of dehydration and cyclization reactions catalyzed by specific (dedicated) lanthionine synthetases, such as LanB and LanC in class I lantibiotics, or by single multifunctional synthetases, such as LanM, LanKC, and LanL described for classes II, III, and IV, respectively (Fig. 1) (reviewed in references 6 and 7). The class I LanB dehydratases are aminoacyl-tRNA dependent, with initial glutamylation of Ser/Thr, followed by glutamate elimination and generation of Dha/Dhb (8, 9). The multifunctional synthetases (LanM, LanKC, and LanL) require (d)NTPs for dehydration to phosphorylate Ser/Thr residues, followed by elimination and generation of Dha/Dhb (10–13). The class II LanMs consist of two domains (an N-terminal dehydratase and a C-terminal cyclase domain), whereas the multifunctional synthetases in classes III and IV have three domains consisting of a N-terminal lyase domain, central kinase domain, and C-terminal cyclase domain (5,

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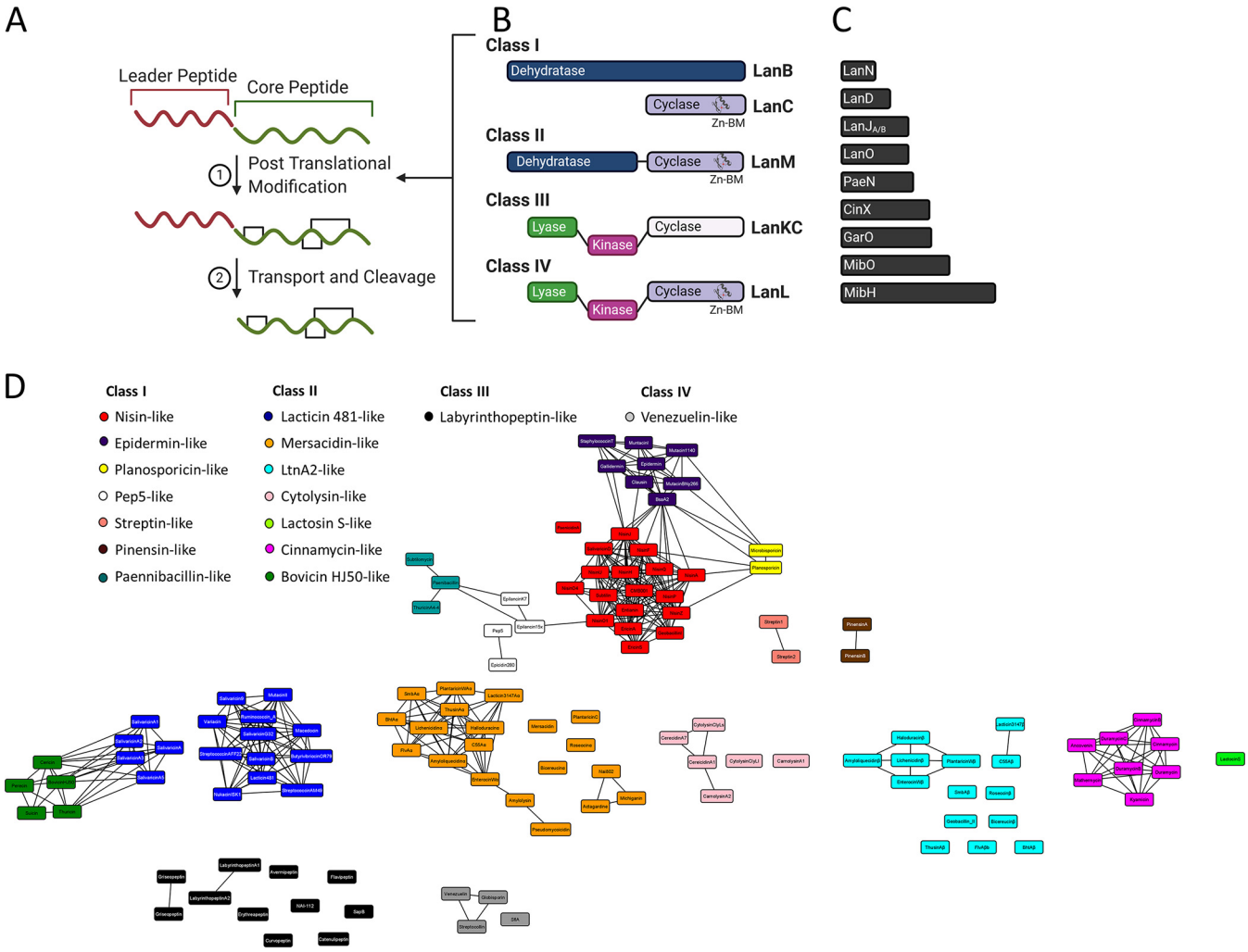

FIG 1 Biosynthesis and classification of lanthipeptides. (A) Generalized scheme of lanthipeptide biosynthesis (precursor peptide made up of leader and core peptides). (B and C) Four main classes of synthetases (B) and additional modification enzymes (C) involved in PTM of lanthipeptides. (D) Sequence similarity network of lanthipeptide core peptides generated with the Enzyme Function Initiative-Enzyme Similarity Tool (EST-EFI [https://efi.igb.illinois.edu/efi-est/]) (E value cutoff, 10^{-3}) and visualized in Cytoscape (v.3.8.0). Abbreviations: LanN, lysinoalanine synthase; LanD, flavin-containing Cys decarboxylase; $LanJ_{A/B}$, dehydrogenase (A indicates Zn^{2+} dependent and B indicates flavin dependent); LanO, oxidoreductase; PaeN, acetylation (paenibacillin); CinX, α-ketoglutarate (α-KG)/iron(II)-dependent hydroxylase (cinnamycin); GarO, flavin-dependent monooxygenase (actagardine); MibO, hydroxylase (microbisporicin); MibH, Trp halogenase (microbisporicin); and Zn-BM, zinc binding motif.

10–13). The N-terminal dehydratase domain of class II synthetases is responsible for the phosphorylation and elimination steps, and in classes III and IV, this is carried out by two different domains, namely, the kinase and lyase domains, respectively. Dehydrated Ser/Thr (i.e., Dha/Dhb) are subsequently cyclized by the addition of a Cys thiol through a Michael-type addition: the resulting enolate intermediate undergoes protonation to form either Lan (Dha-Cys) or MeLan (Dhb-Cys) cross-links (14). The main differentiating factor between class III and class IV synthetases is in their C-terminal cyclase domains, with class IV featuring the conserved zinc-binding domain, also present in class I and II cyclases, while this feature is lacking in class III cyclase representatives (5, 11, 15). Furthermore, in the case of some class III lanthipeptides, the enolate intermediate formed during the first nucleophilic attack is not protonated and undergoes an additional Michael addition, with a second Dha yielding a bicyclic structure termed labionin (Lab) (15, 16). Based on these synthetase differences, lanthipeptides are grouped into four classes, with further division into subclasses based on differences in the amino acid sequences and tertiary structures of the mature lanthipeptides (Fig. 1; Fig. S1 to S4) (3, 17). Lantibiotics may undergo additional posttranslational

TABLE 1 Lanthipeptide producers and applications

Lanthipeptide(s)	Producer strain	Class, subclass	Type tested[a]	Bioactivity (reference[s])
Nisin	*Lactococcus lactis*	I, nisin-like	*In vivo*, *in vitro*, TC	Antimicrobial (160), anticancer (129), immunomodulatory (46)
Subtilin	*Bacillus subtilis*	I, nisin-like	*In vitro*, *in vivo*	Antimicrobial (161, 162)
Ericin	*Bacillus subtilis*	I, nisin-like	*In vitro*	Antimicrobial (163)
CMB001	*Paenibacillus kyungheensis*	I, nisin-like	*In vitro*	Antimicrobial (33)
Gallidermin/epidermin	*Staphylococcus gallinarum*/*S. epidermidis*	I, epidermin-like	*In vitro*, TC	Antimicrobial (164), immunomodulatory (47)
Clausin	*Bacillus clausii*	I, epidermin-like	*In vitro*, *in vivo*	Antimicrobial (27)
Mutacin 1140	*Streptococcus mutans*	I, epidermin-like	*In vitro*, *in vivo*, TC	Antimicrobial (40, 165)
Mutacin B-Ny266	*Streptococcus mutans*	I, epidermin-like	*In vitro*, *in vivo*	Antimicrobial (98, 166)
Planosporicin	*Planispora alba*	I, planosporicin-like	*In vivo*, *in vitro*	Antimicrobial (28, 82)
NAI-107 (microbisporicin)	*Microspora corallina*	I, planosporicin-like	*In vivo*, *in vitro*	Antimicrobial (28, 29)
Mersacidin	*Bacillus amyloliquefaciens*	II, mersacidin-like	*In vitro*, *in vivo*	Antimicrobial (31, 34)
Actagardine	*Actinoplanes liguraie*	II, mersacidin-like	*In vivo*, *in vitro*	Antimicrobial (79, 167)
Duramycin/cinnamycin	*Streptomyces cinnamoneus*	II, cinnamycin-like	*In vitro*, *in vivo*, TC	Antimicrobial (168), antiviral (141, 168), anticancer (135, 136), ion channel regulator (50, 51), immunomodulatory (121)
Lacticin-3147	*Lactococcus lactis*	II, mersacidin-like; II, Ltn2-like	*In vivo*, *in vitro*	Antimicrobial (32, 169)
Amyloliquecidin	*Bacillus amyloliquefaciens*	II, mersacidin-like; II, Ltn2-like	*In vivo*, *in vitro*	Antimicrobial (27)
Pinensins	*Chitinophaga pinensins*	I, pinensin-like	*In vitro*	Antimicrobial (170), antifungal (170)
Labyrinthopeptins	*Actinomadura namibiensis*	III, labyrinthopeptin-like	*In vivo*, *in vitro*, TC	Antiviral (44), antiallodynic (16)
NAI-112	*Actinoplanes* sp. strain DSM 24059	III, labyrinthopeptin-like	*In vivo*, *in vitro*	Antimicrobial (mild) (48), antiallodynic (48), antinociceptive (48)

[a]TC, tissue culture.

modifications (PTMs), such as the introduction of D-amino acids, oxidative decarboxylation of the C terminus, formation of a lysinoalanine ring, and formation of an N-terminal lactate group (Fig. 1) (18–21). This results in the formation of a diverse number of lantibiotics and lanthipeptides. In most cases, the structural genes of lantibiotics form part of a biosynthetic gene cluster and contain the biosynthetic machinery for modification, export, leader processing, and regulation. This was first shown for the lantibiotics nisin (22), epidermin (23), and subtilin (24) and has subsequently been illustrated for numerous other lanthipeptides.

Since the first description of lantibiotics, numerous lantibiotics have been identified and characterized, with nisin, first reported in 1928, being the most well known: nisin has been used as a food preservative for over 50 years, which is currently the only industrial application of a lantibiotic (1, 25, 26). Although the antimicrobial properties of lantibiotics, especially against antibiotic-resistant and clinically relevant strains of *Staphylococcus* (27–35), *Enterococcus* (27–30, 33, 35), and *Clostridium* (28, 36–40) spp., have been reported in several studies (Table 1), very few clinical studies have been published (reviewed in reference 41). This may be changing, as more recent studies have shown that some lantibiotics have broader applications than initially appreciated due to activities in addition to their antibacterial properties, such as antiviral activity (42–45), immunomodulatory properties (46, 47), antiallodynic effects (16, 48), and the ability to alleviate cystic fibrosis (49–54) (Table 1). Lanthipeptide biosynthetic genes are widespread within the genomes of taxonomically distinct bacterial species, providing a substantial repository for peptides with a wide range of potentially novel structures and bioactivities (26). More recent interrogation of the genomes of understudied phyla suggests that lanthipeptides are likely much more diverse than is currently appreciated, and many novel posttranslational modification mechanisms have yet to be described (55). Such genome mining efforts have indicated that lanthipeptide synthetases have been repurposed for production of natural products other than lanthipeptides, thereby expanding natural product diversity. The development of rapid screening methods of large lantibiotic/lanthipeptide libraries further adds to the

discovery pipeline (56–58). With current advances in heterologous gene expression, these peptides may be produced by laboratory strains at high levels (Table 2). Heterologous expression of unusual biosynthetic systems identified through (meta)genome mining efforts could result in the discovery of natural products with new scaffolds that potentially have interesting biological activities.

In this review, we evaluate advances made in heterologous gene expression and report on progress made in the medical application of lanthipeptides.

APPLICATIONS OF LANTHIPEPTIDES

Due to the diversity and complex nature of lantibiotics, they have been explored for use in various medical applications, with some in clinical trials (Table 1). Lantibiotics have mainly been studied for their application as antimicrobials. However, they have bioactivities that extend beyond antimicrobial. Furthermore, their modification machinery can be used to stabilize peptides, improving their *in vivo* efficacy under a variety of conditions, such as stroke and diabetic nephropathy (59, 60). An increase in the number of sequenced genomes, including data from unculturable organisms, has led to an increase in the *in silico* analyses of genomic data that may yet reveal lantibiotics/lanthipeptides with more diverse bioactivities (26). With the help of *in vitro*/*in vivo* engineering, these putative peptides can then be functionally expressed, increasing the arsenal of candidates with potential medical applications. The development of efficient *in vitro*/*in vivo* strategies for screening and expressing these peptides makes large-scale production a realistic possibility.

TREATMENT OF INFECTIONS

Although a substantial arsenal of antibiotics is currently available for the treatment of a wide range of infections, antibiotics are losing efficacy against once treatable infections—a phenomenon accelerated by the incorrect use of antibiotics and the resulting microbial multidrug resistance (61, 62). A pessimistic view may be that we are treating ourselves back into an era without antibiotics. The World Health Organization (WHO) reports the high rates of resistance to antibiotics commonly used to treat serious bacterial infections, and the Centers for Disease Control and Prevention (CDC) estimates that in the United States more than 2.8 million people contract infections that are caused by microorganisms resistant to one or more of the prescribed antibiotics (61, 62). Although antibiotic resistance is on the increase, challenges faced by drug discovery programs have led to an antibiotic discovery void, with the introduction of very few new antibiotic classes in the last 2 decades (63). However, new technologies in bioinformatics, structural and chemical biology, and high-throughput screening techniques can aid in novel antibiotics making it into the drug discovery pipeline.

Lantibiotics are attractive antimicrobials as they are active at low concentrations and mostly target high-value targets, such as lipid II (Fig. 2). The majority of lantibiotics bind to the cell wall precursor lipid II, preventing cell wall biosynthesis and facilitating the disruption of the bacterial membrane (64). As an example, the prototypical lantibiotic nisin binds to the pyrophosphate moiety of lipid II, with its two N-terminal rings crucial for this interaction (65). Formation of the pore complex results in cell membrane permeabilization and dissipation of the proton motive force (64). The ability of certain globular lantibiotics such as epidermin-like lantibiotics to form pores is dependent on membrane thickness. These peptides are much shorter than nisin-like lantibiotics and cannot form pores in cell membranes exceeding 40 Å (66). However, due to their ability to bind to lipid II, they are still able to disrupt cell wall biosynthesis.

Lantibiotics are mainly produced by Gram-positive organisms and as a result are very effective against closely related Gram-positive bacteria, with limited activity against Gram-negative bacteria. This is due to the structural design of the of Gram-negative bacterial outer membrane, which prevents access to the peptidoglycan layer (home of lipid II) and cytoplasmic membrane. Furthermore, the anionic cell surface of Gram-negative bacteria results in binding of the cationic lantibiotics, where such an

TABLE 2 Examples of lanthipeptide heterologous expression systems

Lanthipeptide	Wild type[a]	Class, subclass	Heterologous producer	Vector(s) used[b]	Cleavage[c]	Intracellular/ secreted[d]	Yield[e]	Culture conditions[f]	Reference(s)
Nisin	*L. lactis*	I, nisin-like	*E. coli* BL21(DE3)	pRSF*nisA-nisB* (HispLanA and LanB), pACYC*nisC* (LanC)	*In vitro*, trypsin	IC (SF, ISF)	24 mg/liter (precursor)	TB, induced with IPTG, expressed 15 h at 18°C	149
	L. lactis	I, nisin-like	*E. coli* BL21(DE3)	pRSF*GFPnisA-nisB* (HisGFPpLanA and LanB), pACYC*nisC* (LanC)	*In vitro*, thrombin, hNisP	IC (SF)	1.99 mg/liter (core)	TB, induced with IPTG, expressed 24–48 h at 26°C	151
	L. lactis	I, nisin-like	*L. lactis* NZ9000	pIL3EryBTC (LanB, LanT and LanC), pNZ*nisA* (pLanA)	*In vitro*, hNisP	Secreted	1.7–3.5 mg/liter (core)	MEM, induced with nisin, expressed 3–24 h at 30°C	146, 147, 171
Pep5	*Sta. epidermidis* 5	I, Pep5-like	*E. coli* BL21(DE3)	pRSF*GFPpep5-nisB* (HisGFPpLanA and LanB), pACYC*nisC* (LanC)	*In vitro*, thrombin, hNisP	IC (SF)	ND	TB, induced with IPTG, expressed 24–48 h at 26°C	151
Gallidermin	*Sta. gallinarum* Tu3928	I, epidermin-like	*L. lactis* NZ9000	pIL3EryBTC (LanB, LanT and LanC), pNZE*gdmA* (pLanA), pNZE*gdmD* (LanD)	*In vitro*, trypsin	Secreted	ND	MEM, induced with nisin, expressed 2 h at 30°C	147
NAI-107 (microbisporicin)	*Microbispora* sp. strain 107891	I, planosporicin-like	*E. coli* BL21(DE3)	pET*mibA-mibB* (HispLanA and LanB), pCDF*mibC* (LanC), pACYC*tRNA*^{Glu}*X4-mgltX* (tRNAGlu$_{CUC-1}$ *iso*-acceptor and GluRS)	*In vitro*, LysC	IC (SF)	ND	TB, induced with IPTG, expressed 18–20 h at 25°C	9
Ala(0)-actagardine	*A. liguriae* ATCC 31048	II, mersacidin-like	*E. coli* BL21(DE3)	pRSF*garA-garM* (HispLanA and LanM), pCDF*garO*(2x) (GarO)	*In vitro*, trypsin aminopeptidase A8200	IC (SF)	4.2 mg/liter (precursor)	LB, induced with IPTG, expressed 20 h at 18°C	172
	A. garbadinensis ATCC 31049	II, cinnamycin-like	*S. lividans*	Cosmid CosAG14 (contains *garA* BGC)	*In vivo*	Secreted	50–80 mg/liter (core)	GM1, not induced, expressed 72 h at 30°C	159
Bovicin	*Str. bovis* HJ50	II, bovicin HJ50-like	*E. coli* BL21(DE3) and C43(DE3)	pET28a*bovA* (HispLanA), pET28a*bovA-bovM* (HispLanA and LanM), pACYC*bovT*$_{150}$*M* (truncated HisLanT fused to LanM), pACYC*bovT*$_{150}$ (truncated HisLanT)	*In vitro* and *in vivo*, BovT, BovT150, BovT50M	IC (SF, ISF), secreted	0.2–0.91 μg/ml (core) in cell lysates	LB, induced with IPTG, expressed 20 h at 16°C	155
Carnolysin	*C. maltaromaticum* C2	II, cytolysin-like	*E. coli* BL21(DE3)	pRSF*crnA1-crnM* (HispLan and LanM), pRSF*crnA2-crnM* (HispLan and LanM), pET*crnJ* (LanJ$_B$)	*In vitro*, CrnT150, GluC	IC (SF, ISF)	0.5 mg/liter (precursor)	LB, induced with IPTG, expressed 20 h at 18°C	173
Cinnamycin	*St. cinnamoneus* DSM 40005	II, cinnamycin-like	*E. coli* BL21(DE3)	pRSF*cinA-cinM* (HispLanA and LanM), pACYC*cinX* (CinX)	*In vitro*, LysC		2 mg/liter (precursor)	LB, induced with IPTG, expressed 20 h at 18°C	20
Haloduracin	*B. halodurans* C-125	II, mersacidin-like (Halα); II, LtnA2-like (Halβ)	*E. coli* BL21(DE3)	pRSF*halA-halM1* (HispLanA and LanM), pRSF*halB-halM2* (HispLanA and LanM)	*In vitro*, factor Xa	IC (SF)	1–2 mg/liter (precursors)	LB, induced with IPTG, expressed 18 h at 18°C	149
Lichenicidin	*B. licheniformis* 189	II, mersacidin-like (Bliα); II, LtnA2-like (Bliβ)	*E. coli* BL21 Gold (DE3)	pRSF*TM1A1* (pLanA, LanT, and LanM), pRSF*TPM2A2* (pLanA, LanT, LanP, and LanM)	*In vivo*, LicT, LicP	Secreted	4 mg/liter Bliα (core), 6 mg/liter Bliβ (core)	Medium M, induced by autoinduction/IPTG, expressed 16 h at 30°C	154
Roseocin	*Streptomyces roseosporus* NRRL 11379	II, mersacidin-like (Rosα); II, LtnA2-like (Rosβ)	*E. coli* BL21	pRSF*RosA1-rosM* (HispLanA and LanM), pRSF*RosA2-rosM* (HispLanA and LanM)	*In vitro*, GluC	IC (SF)	4 mg/liter (both α and β; precursor)	LB, induced with IPTG, expressed 24 h at 18°C	174
Nukacin ISK-1	*Sta. warneri* ISK-1	II, lacticin 481-like	*E. coli* BL21(DE3)	pET-*nukAM* (HispLanA and LanM)	*In vitro*, LysC	IC (SF)	1.5 mg/liter (precursor)	2$\times$ YT, induced with IPTG, expressed 20 h at 20°C	150
Prochlorosins (1.7, 2.11, and 3.3)	*Prochlorococcus* sp. strain MIT 9313	II, mersacidin-like	*E. coli* BL21(DE3)	pRSF*procA-procM* (HispLanA and LanM)	*In vitro*, trypsin, LysC, TEV	IC (SF, ISF)	10–35 mg/liter (precursors)	LB, induced with IPTG, expressed 20 h at 18°C	149
Ruminococcin A	*Ruminococcus gnavus* E1	II, lacticin 481-like	*E. coli* BL21(DE3)	pWLEO*grv4-grvM* (HisGFPpLanA and LanM)	*In vitro*, TEV	IC (SF)	6 mg/liter (precursor)	TB, induced with IPTG, expressed 18–24 h at 30°C	152
NAI-112	*Actinoplanes* DSM24059	III, labyrinthopeptin-like	*E. coli* BL21(DE3)	pRSF*AplA* (HispLanA), pACYC*AplKC* (LanKC)	*In vitro*, metalloproteases (AplP)	IC (ISF)	1.25 mg/liter (precursor)	LB, induced with IPTG, expressed 20 h at 16°C	175
Labyrinthopeptins			*S. lividans*		*In vivo*	Secreted			158

(Continued on next page)

TABLE 2 (Continued)

Lanthipeptide	Wild type[a]	Class, subclass	Heterologous producer	Vector(s) used[b]	Cleavage[c]	Intracellular/ secreted[d]	Yield[e]	Culture conditions[f]	Reference(s)
	Actinomadura namibiensis DSM 6313	III, labyrinthopeptin-like		pUW*LabSG2* (pLabyA1; LanKC and ABC transporters),[g] pUW*LabSG6* (pLabyA2; LanKC and ABC transporters)[g]			86 mg/liter LabyA1 (core), 14 mg/liter LabyA2 (core)	YEME, R2YE (LabyA1), and NZ amine (LabyA2), not induced, expressed 384 h at 28°C	
Venezuelin	*Streptomyces venezuelae* ATCC 10712	IV, venezuelin-like	*E. coli* BL21(DE3)	pET28*MBPVenA* (MBPHispVenA), pET28*VenL* (HisVenL and mutants)	*In vitro*, TEV	IC (SF)[h]	ND	LB, induced with IPTG, expressed 2.5–3 h at 37°C	12
Globisporin	*St. globisporus* NRRL B2293	IV, venezuelin-like	*E. coli* BL21(DE3)	pET28*asgbA-sgbL* (LanL and either HisMBPpLanA or HispLanA)	*In vitro*, thrombin, TEV	IC (SF, ISF)	0.3–0.7 mg/liter (precursor)	LB, induced with IPTG, expressed 1 h at 37°C	5
SflA	*Streptomyces* sp. strain NRRL S-1022	IV, venezuelin-like	*E. coli* BL21(DE3)	Plasmid coexpressing SflA (HispLanA) and SflL (LanL)	*In vitro*, metalloproteases (StrS1022-P2 and -P4)	IC (SF)	10 μg/liter (precursor)	TB, induced with IPTG, expressed 18–20 h at 18°C	176
Streptocollin	*St. collinus* Tu 365	IV, venezuelin-like	*St. coelicolor* M1146 and M1152	Cosmid A12-1 (contains *stcA* BGC)	*In vivo*	Secreted	5.4–10 mg/liter (core)	SFM agar, not induced, expressed 240 h at 29°C	124

[a]Strain in which the biosynthetic gene cluster is found.
[b]Vectors used for expression are given, and peptides/proteins expressed from vectors are indicated. pLanA, precursor LanA; HispLanA, His-tagged pLanA; HisGFPpLan, His-tagged GFP fused to pLanA.
[c]hNisP, heterologously produced nisin protease; GluC, glutamyl endopeptidase; TEV, tobacco etch virus protease; LysC, endoproteinase LysC.
[d]IC, intracellular; SF, soluble fraction; ISF, insoluble fraction.
[e]ND, not determined; core, core peptide; precursor, precursor peptide.
[f]LB, Luria-Bertani; TB, Terrific Broth; IPTG, isopropyl-β-D-thiogalactopyranoside; MEM, minimal expression medium; TSB, tryptic soy broth; 2× YT, 2× yeast extract-tryptone; SFM, soya flour-mannitol; YEME, yeast extract-malt extract; R2YE, R2 medium containing yeast extract.
[g]Labyrinthopeptin A1 leader peptide modified (Arg-1 to Met-1) and used for pLabyA1 and to replace the pLabyA2 leader peptide.
[h]Modification performed *in vitro* after cleavage of His-tagged precursor peptide from MBP.

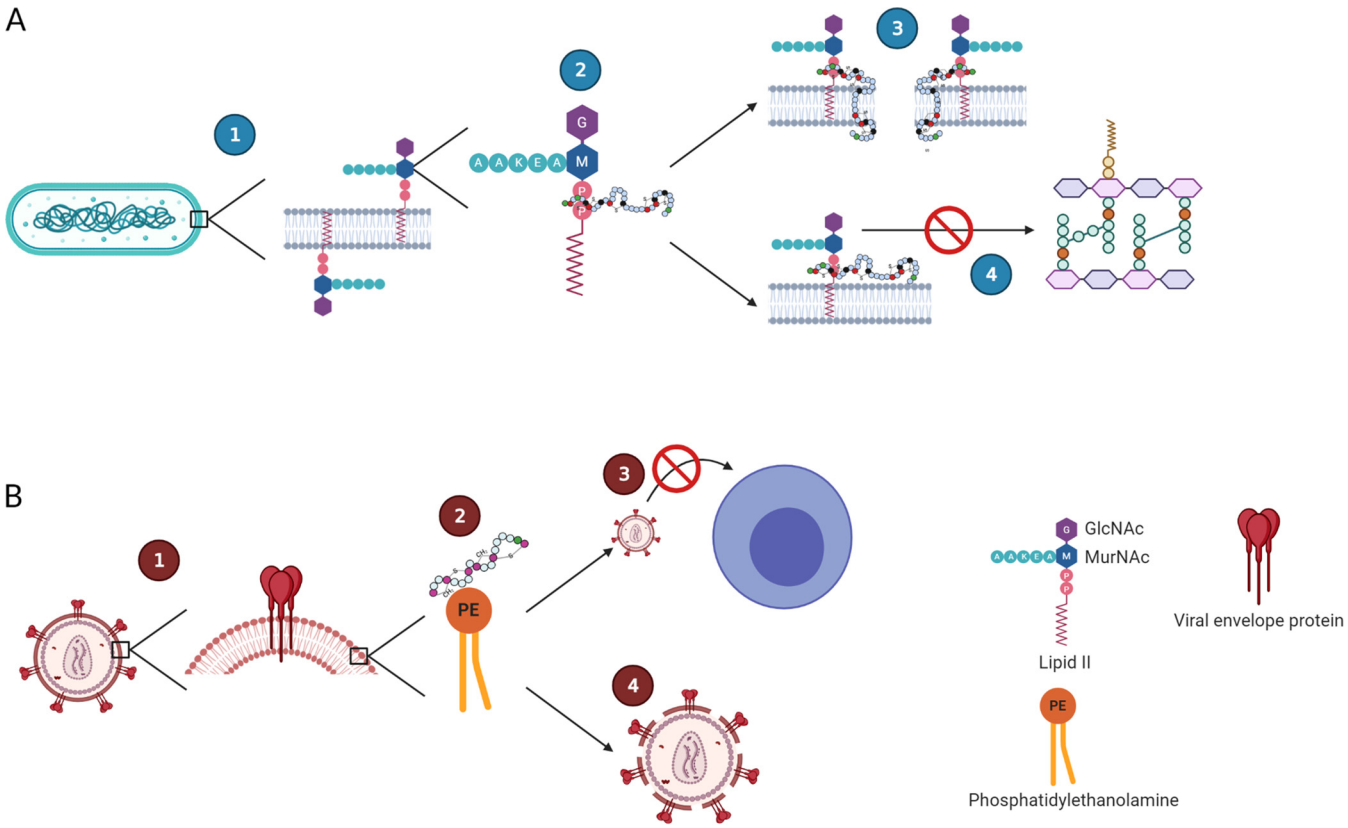

FIG 2 Lanthipeptide antimicrobial and antiviral modes of action. (A) Antimicrobial mode of action of lipid II binding lantibiotics. The peptidoglycan precursor lipid II (1) is the binding site for lipid II binding lantibiotics, which bind to the pyrophosphate cage of lipid II (2). Once bound, the lantibiotics can undergo a conformational change, resulting in insertion into the membrane and subsequent pore formation (3). Additionally, binding of lantibiotics to lipid II can result in inhibition of cell wall biosynthesis (4). (B) Proposed antiviral mechanism of lanthipeptides. Envelope viruses harbor lipids in their virus envelope derived from the host cell membranes, including phosphatidylethanolamine (PE) (1). Lanthipeptides effective against envelope virus bind to PE distributed on the outer virus envelope (2), which can interfere with viral entry into host cells (3) and virolysis (4). The figure was created with BioRender.

interaction potentially increases the stability of the outer membrane through electrostatic interactions (67). This is not surprising considering that most lantibiotics are produced by Gram-positive bacteria. Despite this, lantibiotics have shown promising activity against several of the pathogens on the WHO priority list (63). As such, lantibiotics have been exhaustively tested *in vitro* against various bacteria, with several also tested for their *in vivo* efficacy (Table 1). This review discusses some of these examples regarding lantibiotic antimicrobial activity and other potential bioactivities to illustrate the versatility of lantibiotics/lanthipeptides in their application for human health.

LANTIBIOTICS AGAINST GRAM-POSITIVE BACTERIA

Staphylococci and streptococci. Some of the most promising *in vivo* antimicrobial results for lantibiotics have been obtained against staphylococci. Staphylococci represent one of the main genera represented on the skin, either as commensals or pathogens, with *Staphylococcus aureus* most commonly presenting as a pathogen. Unsurprisingly, the vast majority of skin and soft tissue infections (SSTIs) are caused by *S. aureus* and are usually associated with boils, abscesses, carbuncles and localized wound sepsis (68).

Several lantibiotics are effective against even antibiotic-resistant *S. aureus in vitro* and *in vivo* (27–34, 69). However, there are limited published studies evaluating lantibiotics as topical treatments. A relatively recent study used a promising system comprising the incorporation of nisin into nanofibers (69). This approach proved to be effective in the treatment and prevention of *S. aureus* in mice. After 7 days (with dressings changed on days 2 and 4), the nisin-eluting nanofibers reduced the amount of viable

S. aureus in wounds to 4.3×10^2 CFU/wound, compared to 2.2×10^7 CFU/wound for the control nanofibers. The nisin-eluting and control nanofibers also had a positive effect on wound healing and closure compared to the gauze control, with up to 90% wound closure compared to 74% closure, respectively. The use of nanofibers as a delivery vehicle for lantibiotics has potential as it provides controlled release over a prolonged period as well as provides a scaffold for improved wound healing. In another study, nisin, clausin, and the two-component lantibiotic amyloliquecidin were shown to be just as effective as a commercially available antimicrobial (mupirocin) in the treatment of *S. aureus*-induced skin infections *in vivo* (27). Lantibiotics were administered as polyvinyl alcohol formulations and along with the mupirocin control were able to significantly reduce the viable *S. aureus* numbers in wounds to 2.1×10^4 CFU/wound for mupirocin, 6.5×10^4 CFU/wound for amyloliquecidin, and 1.6×10^5 CFU/wound for nisin and clausin after 7 days (treatments on days 2, 4, and 6) compared to the control treatment (1.4×10^7 CFU/wound). In this study, the positive effect on wound closure was also observed, indicating a beneficial effect, potentially involving the activation of the hosts' immune system (46, 47). An alternative mechanism for the delivery of lantibiotics may be via administration of an organism producing them as a probiotic. It is hypothesized that lantibiotics produced by commensal bacteria may play a role in maintaining microbial balance, through direct antimicrobial activity (reviewed in reference 70). This was demonstrated, to some extent, using lantibiotic-producing staphylococcal commensals to provide resistance against colonization by *S. aureus* (71). The authors identified antimicrobial-producing coagulase-negative *Staphylococcus* (CoNS) isolates collected from healthy human skin. These isolates produced known lantibiotics, including epidermin and Pep5, as well as the novel lantibiotics *Sh*-lantibiotic-α and *Sh*-lantibiotic-β produced by *Staphylococcus hominis* A9. Using this lantibiotic-producing strain, complete eradication of *S. aureus* colonized on the backs of mice could be achieved after twice-daily applications for 1 week. Furthermore, these lantibiotics were shown to synergize with the human cationic peptide LL-37, increasing activity against *S. aureus* 16- and 32-fold for *Sh*-lantibiotic-α and *Sh*-lantibiotic-β, respectively. To further investigate the potential of CoNS to treat skin diseases associated with *S. aureus*, such as atopic dermatitis (AD), the authors used formulations of antimicrobial-producing CoNS in autologous microbiome skin transplants. Using their formulation, the amount of *S. aureus* that could be isolated from the skin of AD patients was significantly reduced after 24 h. These results illustrate that the use of lantibiotic-producing strains for autologous microbiome skin transplants holds potential and should be investigated further. However, although the lantibiotic-producing capability of commensal staphylococci may provide a host benefit, the opposite may also be true for pathogenic strains. An example is growth attenuation of *S. aureus* in a mouse abscess model through the disruption of the lantibiotic gene, indicating a potential role in pathogenesis (72). In both of these cases, antimicrobial activity undoubtedly plays a role in terms of competitive exclusion: their potential immune-regulatory roles cannot be overlooked.

In addition to their application in the treatment of topical *S. aureus* infections, several lantibiotics have shown potential for use in other applications related to staphylococcal infections. This includes both preventative and therapeutic approaches to protect against *Staphylococcus* colonization and formation of biofilms on medical devices, such as catheters, cardiac devices, and prosthetic implants, which can complicate treatment (73–75).

Lantibiotics are susceptible to low bioavailability when used systemically: perhaps due to the ability of some to activate the immune system, resulting in rapid degradation/inactivation *in vivo*, or the binding to host components (29, 40, 47, 76). Despite this, some success has been reported for the systemic use of lantibiotics for the treatment of infections. For example, the class I lantibiotic microbisporicin (NAI-107) has shown promise, with equivalent or superior activity compared to reference treatments (e.g., penicillin, vancomycin, and linezolid) in methicillin-resistant *S. aureus* (MRSA) and

glycopeptide-intermediate *S. aureus* infections (28, 29). In a rat granuloma pouch model, two 20-mg/kg doses (at 12 or 24 h) of microbisporicin, administered directly after infection with MRSA, resulted in a 3-log reduction in bacterial cell counts after 72 h, with no regrowth up to 96 h (29). Similar results were also obtained in a rat endocarditis model, and in both models, microbisporicin performed similar to or better than treatment with 100 mg/kg vancomycin (29). Interestingly, microbisporicin was more effective when administered intravenously compared to subcutaneously (1.5 to 2.5 times higher), suggesting that not all lantibiotics are equally capable of crossing host membrane or tissue barriers (29, 76). Similarly, the class II lantibiotic mersacidin has also shown potential with superior activity over vancomycin against MRSA in an *in vivo* infection model (50% effective doses [ED_{50}s] of 2.59 to 10.81 mg/kg of body weight and 7.20 to 18.98 mg/kg, respectively) where mice were injected intraperitoneally with lethal amounts of *S. aureus* (MRSA and methicillin-sensitive *S. aureus*) and treated subcutaneously (34). Mersacidin also has reduced bioavailability when injected subcutaneously, which only improves after using the more water-soluble potassium mersacidin (34). This supports our interpretation that there may be an interaction between the peptide and host components when administered into tissue rather than intravenously. A combination of mutacin 1140 analogs have recently been used to improve the efficacy of lantibiotics in the treatment of systemic *S. aureus* infection (77). The two analogs had substitutions at positions 2 (K2A) and 13 (R13A) resulting in improved activity and stability (77). The two analogs were more active than native mutacin, with 2- and 8-fold increases in activity (MIC) against MRSA (*S. aureus* ATCC 33591) for K2A and R13A, respectively, compared to the native peptide. *In vivo* stability was also increased, with up to 4.1- and 5.7-fold higher peak plasma concentrations (K2A and R13A variants, respectively) compared to the native mutacin 1140 after 60 min (administered intravenously at 10 mg/kg in mice). Further investigation revealed that the K2A mutacin exhibited the lowest clearance levels and highest AUC (area under the concentration-time curve), while R13A had the longest half-life and highest V_{ss} (volume of distribution at steady state) *in vivo*. Efficacy against MRSA in a murine systemic infection model also proved promising, with intravenous administration of the combined analogs (1:1 ratio at 10 mg/kg, with 5 mg/kg of each analog), resulting in 100% survival of animals after 5 days, compared to 100% mortality in the vehicle group. Furthermore, bacterial loads in the liver and kidneys were significantly reduced compared to the vehicle group. Using a combination of either different lantibiotics or analogs with distinct pharmacokinetic and activity profiles can be advantageous, as this can increase both efficacy as well as antimicrobial spectrum *in vivo*.

Several lantibiotics have also proved effective against pathogenic streptococci. Streptococci include several pathogenic strains and are divided into alpha- and beta-hemolytic streptococci. Alpha-hemolytic streptococci includes *Streptococcus pneumoniae*, which is the cause of pneumococcal infections, including otitis media, sinusitis, pneumonia, and meningitis. Mice infected intraperitoneally with *S. pneumoniae*, at concentrations sufficient to result in death, were treated with either nisin or vancomycin (78). Two intravenous treatments with nisin (0.16 mg/kg) resulted in survival of all animals, whereas the survival of mice treated with vancomycin was only 83% when treated with 1.25 mg/kg, and 100% survival was only achieved after treatment with 2.5 or 5.0 mg/kg. Nisin had low blood and tissue levels (serum half-life of 0.9 h), but this was still sufficient to prevent death of the mice (78). Like microbisporicin, nisin was also more effective when administered intravenously (0.16 versus 2.5 mg/kg for 100% survival). In another study, carboxamides of actagardine were generated (79). The monocarboxamides were more active than other variants *in vitro*, and a more water-soluble derivative was effective in a murine septicemia model, with effective dose values (ED_{50}, 0.23 to 3.5 mg/kg/day) comparable to those of the reference antibiotics used (0.03 to 26 mg/kg/day) (79). Compared to nisin, the actagardine derivative was, however, eliminated faster, with a serum half-life of 0.3 h (78). Microbisporicin also proved

highly effective against *S. pneumonia*, with ED_{50} values lower than those of linezolid (0.51 versus 15.9 mg/kg, respectively) (29).

Beta-hemolytic streptococci are divided into groups A and B. Group A streptococci are found on the skin and inside the throat and are responsible for most beta-hemolytic streptococcal infections. Common infections caused by group A beta-hemolytic streptococci (GAS [*Streptococcus pyogenes*]) include impetigo, cellulitis, pharyngitis, and scarlet fever. Several lantibiotics, including microbisporicin, mersacidin, actagardine, and lantibiotics produced by streptococci (e.g., salivaricin 9 and streptin) are active against GAS (80, 81). The same water-soluble derivatives of mersacidin and actagardine successfully used against *S. aureus* and *S. pneumoniae* also proved effective in the treatment of *S. pyogenes in vivo* (34, 79). Planosporicin (ED_{50}, 3.75 mg/kg) has also been reported to be effective in preventing septicemia caused by *S. pyogenes* in mice when administered intravenously or subcutaneously (82).

Enterococci. Enterococcal infections, and specifically those caused by vancomycin-resistant enterococci (VRE), are characterized as a serious threat by the CDC and resulted in 54,500 cases (5,400 deaths) in the United States in 2017 (61). Lantibiotics, including lacticin 3147, nisin, mersacidin, epidermin, haloduracin, amyloliquecidin, clausin, and microbisporicin, have promising *in vitro* activity against enterococci, including drug-resistant strains (27–30, 33, 35). *In vivo* efficacy in mice has also been illustrated for microbisporicin, where intravenous and subcutaneous administration exhibited the lowest ED_{50} values (2.3 and 2.8 mg/kg) against two VRE strains compared to linezolid (5.1 and 22.4 mg/kg) (29). Recently, a novel approach using synthetic biology was used to generate a peptide with two lipid II binding motifs (N-terminal domain of nisin and C-terminal domain of haloduracin α) (83). This variant, termed TL19, displayed increased activity (MIC, 0.9 to 15 μM) against several multidrug-resistant (MDR) *Enterococcus faecium* strains *in vitro* compared to nisin (MIC, 1.9 to 3.8 μM) and the C-terminally truncated nisin(1–22) variant (MIC, 30 to 60 μM). Although this variant does not form pores, the additional lipid II binding motif is sufficient to counteract this. Using synthetic biology approaches such as this can be invaluable to generate more effective and stable lantibiotics.

Similar to lantibiotic-producing strains effective against *S. aureus* on skin, lantibiotic-producing probiotics have also shown promise in inhibiting VRE colonization of the gastrointestinal tract (GIT) (84). Kim et al. (84) used a four-strain formulation consisting of *Clostridium bolteae*, *Blautia producta* (nisin-like lantibiotic producer), *Bacteroides sartorii*, and *Parabacteroides distasonis* and reported significant reductions in VRE growth in the gastrointestinal tract of mice compared to the controls (phosphate-buffered saline [PBS] and consortia without a lantibiotic producer). Interestingly, the use of consortia was found to be essential for colonization of the lantibiotic-producing *Blautia producta* strain (85). In the same study, Kim et al. also showed that microbiomes with a high abundance of lantibiotic genes were associated with a lower abundance of *Enterococcus faecium* (84). Furthermore, colonization of germfree mice with microbiomes containing a great abundance of lantibiotic genes resulted in significantly smaller amounts of VRE compared to microbiomes with a low abundance of lantibiotic genes. These results further illustrate the potential of using lantibiotic-producing strains as a delivery vehicle for the treatment or prevention of diseases caused by pathogenic bacteria.

Although VRE is a threat, the emergence of carbapenem-resistant enterococci (CRE)—which are resistant to all treatments evaluated to date—is even more serious (61). To our knowledge, lantibiotics have not been specifically tested against CRE, but given the beneficial effects reported in the context of VRE, investigation of lantibiotic activity against these strains is warranted.

Clostridia. An important foodborne pathogen, involved in severe GIT infections, is *Clostridium difficile*. *Clostridium difficile*-associated diarrhea (CDAD) is one of the major causes of hospital-associated diarrhea, with more than 220,000 hospitalizations in 2017 in the United States alone, with at least $1 billion in excess medical costs (61). Current treatment of CDAD includes oral administration of vancomycin and metronidazole;

however, vancomycin treatment can lead to secondary colonization of VRE in the GIT or even the spread of vancomycin resistance within a hospital environment. Several lantibiotics are effective against *C. difficile*, with some preventing spore outgrowth (28, 36–40). Prevention of spore outgrowth can help in curbing the growth and spread of *C. difficile*, which may contribute to the successful treatment of CDAD. Actagardine (NVB-302) is currently being developed for treatment of *C. difficile* and has successfully completed phase I clinical trials. In an *in vitro* GIT infection model, actagardine compared well with vancomycin in the treatment of *C. difficile*, with less deleterious effects on *Bacteriodes fragilis* (a GIT commensal) (38). Combination treatment with actagardine and ramoplanin was especially effective against multiple *C. difficile* strains (39). The two-component lantibiotic lacticin 3147 has also shown *in vitro* potential for use as a treatment for CDAD (36). In a fecal fermentation model, it completely eliminates *C. difficile* (36). However, in a porcine model, neither of the lacticin 3147 peptides could be detected in digesta of pigs 2 h after oral administration (86). Use of the producing strain has also been investigated (87). While the strain was capable of surviving passage through the GIT, no lacticin 3147 or antimicrobial activity could be detected in the feces of pigs, with the producer strain also incapable of preventing *Listeria monocytogenes* infection in mice. This further illustrates a potential pitfall of using a peptide antibiotic, which may be prone to proteolytic degradation. Degradation can potentially be addressed by encapsulation of the peptides or by using a genetically tailored probiotic strain overexpressing the peptide (69, 88, 89). Additionally, stability is an important factor to consider when evaluating two-component lantibiotics for therapeutic use, as this can be different for the respective peptides.

More recently, promising *in vivo* results have been reported for various variants of mutacin 1140 (40, 89, 90). Of specific interest is variant OG716 (amino acid substitutions Phe1Val and Arg13Asn), showing superior activity against *C. difficile* in hamster models of CDAD (90, 91). Oral administration of OG716 three times a day (days 2 to 5) resulted in 100% survival of animals, with *C. difficile* spore and toxin levels near or below detection limits. Furthermore, considering the sizes of OG716 (2.2 kDa) and vancomycin (1.4 kDa), the ED_{50} values of OG716 are very promising (10.97 and 13.3 μmol/kg/day for OG716 and vancomycin, respectively) (91). Importantly, the various mutacin 1140 variants had low toxicity against human cell lines and in animal models (40, 89, 91). As mentioned earlier, the stability of these peptides in the GIT is an important aspect to consider, and although amino acid substitutions resulted in increased stability of mutacin 1140 variants, they are still susceptible to proteolytic degradation (40, 90). To address this, the use of target-specific enteric-coated capsules is currently being investigated (89).

Mycobacteria. *Mycobacterium tuberculosis* is the causative agent of the respiratory tract infection known as tuberculosis. Worldwide an estimated 10 million people have contracted the disease, with a fatality rate of 11 to 15% (92). Cases of multiple- and extensively-drug-resistant *M. tuberculosis* place an immense burden on the efforts to try and control the spread of *M. tuberculosis*, especially in developing countries (92). The unique cell wall and slow-growth nature of *M. tuberculosis* may make it difficult for lantibiotics (and other treatments) to exert antimicrobial activity. However, the ability of lantibiotics to bind to lipid II gives them an advantage over treatments such as rifampin, which need to be transported across the plasma membrane. The lipid II structure of mycobacteria does differ from those of other bacteria, due to modifications on both *N*-acetylmuramic acid (MurNAc) and the peptide side chain (93). Despite these differences, nisin has activity against the nonpathogenic mycobacteria *M. smegmatis* and *M. bovis*, with intracellular ATP leakage and dissipation of the proton motive force: additionally, hinge mutants were shown to have enhanced activity (94, 95). Nisin, CMB001, and lacticin 3147 are also active against clinical mycobacterial isolates *in vitro*, with lacticin 3147 (MIC_{90}, 7.5 μg/ml) and CMB001 (MIC, 0.3 μg/ml) showing the best activity against *M. tuberculosis* (33, 96). Although these lantibiotics have potential, *in vivo* studies are still required, and an appropriate delivery system still needs to be

developed to reach *M. tuberculosis* residing within tissues. For example, in the context of *M. tuberculosis*-infected macrophages in the distal lung, promising results have been reported for the *in vivo* efficacy of class IIa bacteriocins complexed with phosphatidylcholine-cardiolipin liposomes (97). Given the rise of drug-resistant mycobacteria, further research is warranted to establish the feasibility and use of lantibiotics as an antimycobacterial treatment.

LANTIBIOTIC ACTIVITY AGAINST GRAM-NEGATIVE BACTERIA

Lantibiotics are not particularly active against Gram-negative bacteria, mainly due to the inability of the peptides to cross the outer membrane of these organisms, but several reports of their limited antimicrobial activity are available. This includes activity against *Helicobacter pylori*, *Neisseria meningitidis*, *Neisseria gonorrhoeae*, *Haemophilus influenzae*, *Campylobacter jejuni*, *Serratia marcescens*, *Proteus vulgaris*, and *Escherichia coli* (98–100). Activity against Gram-negative bacteria has been shown to be improved by combination with colistin (polymyxin E) or addition of chelating agents such as EDTA (30, 99). Recently a nisin-like lantibiotic (CMB001) with a similar structure to subtilin was shown to have *in vitro* activity (10 to 15 μg/ml) against MDR *Acinetobacter baumannii* (33). Furthermore, studies have also shown that lantibiotic (specifically nisin) fusion with peptides capable of penetrating Gram-negative outer membranes results in increased potency (100). Addition of these penetrating peptides to the C-terminal domain of nisin resulted in a significant improvement (up to 12-fold) in activity against several MDR Gram-negative pathogenic bacteria (100). The potential of bioengineering lantibiotics with increased activity against Gram-negative pathogens may further expand the antimicrobial arsenal of lantibiotics. Additionally, the immunomodulatory properties of some lantibiotics can also result in their indirect activity against Gram-negative pathogens (47).

RESISTANCE AGAINST LANTIBIOTICS

The discovery of novel antibiotics is limited by several steps, including target selection, which is important if a novel antibiotic is to remain effective for a prolonged period after administration. In this respect, several lantibiotics target cell wall components, including lipid II, and/or possess dual modes of action. Despite this, lantibiotics, as with any antibiotic, are still susceptible to the development of resistance, and this is an important aspect to consider when evaluating their application as antimicrobial therapies. Changes in the cell wall or alterations in membrane composition are some of the major mechanisms employed by target organisms to protect themselves against lantibiotics.

The cationic nature of lantibiotics is a crucial element in their initial interaction with susceptible bacteria; as such, any change in their charge or the charge of their membrane targets would result in altered attraction. Bacterial membranes are overall negatively charged, and in the case of Gram-positive bacteria, this is because of teichoic acids in their cell wall (101). Due to this anionic nature, cationic antimicrobial peptides (ctAMPs), including lantibiotics, are attracted to the bacterial cell wall. The *dltA* operon, found in numerous bacteria, has been identified as an innate defense against lantibiotics (102–104). The different genes in this operon are responsible for the D-alanylation of teichoic acids, resulting in the incorporation of positive charges and consequentially alternating the electrostatic interaction of ctAMPs with the cells (102, 104). Additionally, it has been proposed that D-alanylation of lipoteichoic acids decreases the flexibility and permeability of the cell wall, protecting the host from ctAMPs (105).

Alterations in the lipid composition of the bacterial cell membrane can also affect the efficacy of lantibiotics. Nisin has been shown to have high affinity for anionic phospholipids (106), such as phosphatidylglycerol and diphosphatidylglycerol, which coincidentally are the most common phospholipids in bacterial membranes (reviewed in reference 107). Changes in the anionic nature of phospholipids in the bacterial

membrane would influence resistance. The phosphatidylglycerol lysyltransferase MprF is a mechanism employed by bacteria to alter their membrane charge through alteration of phosphatidylglycerol (108). This protein catalyzes the transfer of a lysine residue from lysyl-tRNAs to phosphatidylglycerol resulting in lysylphosphatidylglycerol. The addition of positively charged L-lysine can subsequently result in the repulsion of ctAMPs, including lantibiotics (108).

Another interesting mode of resistance is that of immune mimicry, whereby resistant target strains harbor an immunity protein (LanI) or immune-specific ABC transporter [LanFE(G)] that confers immunity to a specific lantibiotic (109). Immunity proteins (LanI) and immune-specific ABC transporters [LanFE(G)] are present in most lantibiotic operons and provide protection to the host from its own mature lantibiotic. The immunity protein LanI is a cell-associated lipoprotein and acts by intercepting the mature lantibiotic, rendering it inert (109, 110). The ABC transporters LanFE(G) act through transporting mature lantibiotics from the membrane, thereby preventing pore formation (109). Usually cross-immunity is rare as these immunity elements are specific to a certain lantibiotic. However, it has been shown that cross-immunity is possible, even in strains that do not harbor lantibiotic biosynthetic genes (111). Additionally, more nonspecific ABC transporters have also been identified that are capable of conferring resistance (112, 113). In general, these transporters can be grouped as either CprABC- or BceAB-type transporter families (reviewed in reference 114). These observations are concerning as these immunity elements can also potentially be horizontally transferred, resulting in the increased resistance to lantibiotics.

Resistance mechanisms are especially important to consider as lantibiotics migrate into the clinical setting, where such resistance can be devastating. For additional information on these and other lantibiotic resistance mechanisms, the reader is referred to the comprehensive reviews by Clemens et al. from 2018 (114) and Draper et al. from 2015 (115).

OTHER THERAPEUTIC APPLICATIONS OF LANTIBIOTICS AND LANTHIPEPTIDES

In addition to the antimicrobial potential of lantibiotics, lanthipeptides (including lantibiotics) have shown a diverse range of bioactivities. This is likely due to the diverse nature of these peptides and the unique properties that the various PTMs can confer. Examples are presented below to illustrate the diversity of lantibiotic bioactivity.

Immune modulation. Several ctAMPs (such as LL-37 and α- and β-defensins) play a crucial role in modulating the immune system during infection and injury (116). Most complex species have ctAMPs, which interact with the innate immune system and are generally short overall and positively charged, with a large proportion of hydrophobic residues, making them very similar to lanthipeptides.

Lantibiotics have shown immune-regulatory properties, with nisin, gallidermin, and Pep5 being capable of inducing the release of multiple chemokines at levels similar to that of LL-37, with nisin modulating multiple signaling pathways (47). The protective effect as a result of the immune-modulatory properties of nisin pretreatment provides effective protection for mice infected with *Salmonella enterica* serovar Typhimurium and *E. coli* (47). In line with this interpretation, nisin has also been shown to activate neutrophil release of neutrophil extracellular traps (NETs), a mechanism used by neutrophils to trap, contain, and kill pathogenic organisms and which is thought to be particularly important in the immune response to pathogens too large to be destroyed by phagocytosis alone (46), as reviewed in reference 117. The formation of NETs may thus—apart from contributing to the reduced loads of *S.* Typhimurium and *E. coli* reported in mice pretreated with nisin—also contribute to immune activity against larger pathogens, such as fungi (118). Although the antimicrobial properties associated with NET formation can be advantageous, chronic NET formation, which entails cellular release of large amounts of free radicals as well as nuclear material such as histones, is also associated with chronic inflammation and increased risk for autoimmune diseases, such as rheumatoid arthritis (reviewed in reference 119). This should be considered

when evaluating nisin (or potentially other lanthipeptides) as a chronic therapeutic strategy. In addition to effects on the innate immune system, nisin has also been shown to have effects on the adaptive response in mice (120). This was demonstrated through the addition of nisaplin (a commercially available nisin preparation) to the feed of mice, resulting in the short-term increase of $CD4^+$ and $CD8^+$ cells and T lymphocytes. The nature and significance of this modulation remain to be further elucidated.

Other immune-regulatory properties of lantibiotics come in the form of indirect modulation of phospholipase A2. Phospholipase A2 plays an important role in inflammatory responses, resulting from its role in the release of arachidonic acid. The oxidative metabolism of arachidonic acid results in eicosanoids, such as prostaglandins and leukotrienes, which are strong mediators of the immune system. Cinnamycin-like lantibiotics can indirectly inactivate phospholipase A2 by sequestering phosphatidylethanolamine (PE; substrate for phospholipase A2), thereby having the potential to indirectly mediate inflammatory responses (121). The cinnamycin-like lantibiotic ancovenin is also an inhibitor of angiotensin-converting enzyme (ACE) (122), which is responsible for the poor stability of angiotensin II in circulation and which has been implicated in hypertension and diabetic inflammation and fibrosis (123). On the topic of ACE inhibition and maintenance of angiotensin II function, peptidase-resistant lanthionine-stabilized angiotensin-(1–7), was recently shown to confer benefit in the context of experimental diabetic nephropathy and cerebral stroke (59, 60). Interestingly, streptocollin, which has a similar structure to cinnamycin-like lantibiotics (although forming part of the venezuelin-like class IV lanthipeptides) (Fig. S2G and S4) is unable to inhibit phospholipase A2, although its PE binding has yet to be evaluated (124). This peptide is, however, able to partially inhibit protein tyrosine phosphatase 1B (PTP1B). Protein tyrosine phosphatase 1B is a regulator of various signaling pathways and is best known for its role in insulin signaling, but it also has roles in immune cell signaling (125). Inhibition of PTP1B has been identified as a potential strategy to improve insulin sensitivity and also has therapeutic potential in the treatment of Alzheimer's disease and diabetes (126).

These results suggest that lantibiotics can interact and modulate the immune system, potentially using similar mechanisms employed by human and other ctAMPs. Additional cell biology research is required to fully understand how lantibiotics/lanthipeptides interact with cells of the immune system. This will give further insight into the roles these peptides might play in host-microbe interaction and potential therapeutic application.

Neuropathic pain relief. Labyrinthopeptin-like lantibiotics have limited antibacterial activity. However, labyrinthopeptin A2 and NAI-112 have been shown to have antiallodynic and antinociceptive properties in mice (16, 48). Labyrinthopeptin A2 administered intravenously at concentrations ranging from 0.01 to 3.0 mg/kg resulted in significant attenuation of tactile allodynia (ED_{50}, 50 μg/kg). Efficacy remained stable over 6 h posttreatment, with loss of efficacy after 24 h (16). Similarly, NAI-112 was also able to significantly reduce allodynia and hyperalgesia 2 h after administration, albeit at much higher doses ($>$10 mg/kg) (48). Significant antinociceptive effects could be observed at somewhat lower doses (from 3 mg/kg). At this point, due to differences in experimental procedures, the efficacies of these two lanthipeptides cannot be directly compared. More research is warranted in this context, as the mechanisms of action of these peptides have not been elucidated, although a potential interaction with the vanilloid pathway has been suggested for NAI-112 (48).

Ion channel regulation. Duramycin has potential in the treatment of cystic fibrosis, which is caused by abnormal chloride ion transport into cells. It has been demonstrated in tissue culture that the efflux of chloride observed after duramycin treatment is associated with a decrease in intracellular calcium levels (49). It was therefore proposed that the efflux of chloride from epithelial cells is likely due to the interaction duramycin has with cell membranes. This is supported by the interaction that duramycin has with PE, whereby it can be deposited into the cell membrane and indirectly affect ion channel function (49–51). Duramycin has undergone phase I and II clinical

trials, with phase II clinical trials reporting it to be safe, with overall positive results on the pulmonary function of cystic fibrosis patients (52–54). This effect of duramycin to lower cellular calcium levels may also have broader application in health, given the known association between intracellular accumulation of calcium and a variety of diseases linked to cumulative oxidative damage and chronic inflammation, such as neurodegenerative disease, cancer, accelerated aging, and type II diabetes (reviewed in reference 127).

Anticancer treatment. Nisin (nisins A and Z) has been shown to be effective *in vitro* and *in vivo* against head and neck squamous cell carcinoma (HNSCC) (128, 129). Nisin appears to preferentially induce apoptosis in HNSCC cells in a dose-dependent manner, with minimal effect on primary keratinocytes. This may be due to the structural differences in the plasma membrane, specifically the phospholipid content, of the different cell types. This is supported by the observation that nisin binds to phosphatidylcholine, which is known to be increased in cancer cells (along with PE) (reviewed in references 130 to 132). The mechanism by which nisin induces apoptosis has been proposed to be calcium dependent (influx of calcium) (129). The subsequent influx of calcium results in the activation of calpain-1, resulting in caspase 3-independent apoptosis (128). This is further supported by the observation that nisin affects plasma membrane integrity through the release of lactose dehydrogenase (LDH) (76). Additionally, the potency of nisin *in vitro* and *in vivo* can be further increased in combination with 5-fluorouracil or doxorubicin (133, 134). The involvement of calcium in this context and the increased efficacy by addition of doxorubicin, which is known to exert anticancer effects via induction of free radical damage in cancer cells, again suggest that lantibiotics may have a role in the modulation of redox status, although the nature of this modulation varies between lantibiotics.

Duramycin has also shown potential for the treatment of cancer and has been shown to induce apoptosis and reduce proliferation in tumor cells (135, 136). Furthermore, due to its high affinity for PE, duramycin can be more selective toward cancerous cells. An interesting application of duramycin as an anticancer treatment is its fusion to IgG, generating a new duramycin-IgG variant (137, 138). Fusion of IgG to duramycin does not influence its PE binding capability and has the advantage of reducing duramycin cytotoxicity. The IgG fused to duramycin helps guide the host immune cells to apoptotic cells, resulting in enhanced phagocytosis. Furthermore, tumor growth (MethA tumors) is inhibited in mice after treatment with duramycin-IgG (138). Since duramycin binds to PE and the Fc region on (fused) IgG antibodies interacts with phagocytic cells to enhance phagocytosis, duramycin is likely cleared from the site effectively soon after inducing apoptosis in cancer cells, via phagocytosis, which would explain its lower cytotoxicity to surrounding normal cells.

The urokinase plasminogen activator (uPA) is a serine protease responsible for the conversion of plasminogen to plasmin. The urokinase plasminogen activator system has been implicated in activities associated with tumor progression and metastasis and has been identified as a potential target for anticancer therapy (reviewed in reference 139). Using a phage display system, Urban et al. screened a lanthipeptide library for peptides capable of binding to uPA (57). Using this system, they identified several novel lanthipeptides capable of inhibiting the catalytic ability of uPA. The application of these peptides was not specifically evaluated for their anticancer capabilities, but they do show potential. More importantly, this study has highlighted techniques that could vastly increase the efficiency with which potential candidates may be screened for anticancer activity or, in fact, other bioactivities.

Antiviral capacity. Various lantibiotics, including nisin, labyrinthopeptin, and duramycin, have been evaluated for their antiviral properties (42–45, 140, 141). Of interest are the antiviral properties of labyrinthopeptin A1 and duramycin, which show antiviral activity through their ability to bind PE (Fig. 2). The PE binding capability of duramycin has proven useful once again, with duramycin being able to inhibit the entry of filo- and flaviviruses into host cells (42, 141). Phosphatidylethanolamine is a ligand for the T-cell Ig mucin domain (TIM) protein TIM1, and together they are involved in

phosphatidylserine (PS)-dependent phagocytosis of apoptotic cells (42). Additionally, TIM family proteins have also been shown to promote infection of enveloped viruses as a result of virion lipid content, specifically PS and PE (42). Duramycin was therefore evaluated for its ability to inhibit TIM1-mediated virus entry through blocking virus attachment to TIM1 (42). Duramycin was reported to be most effective at the entry phase of the viral infection, with no effect observed when duramycin was administered postinfection, and was effective in inhibiting viral entry into human TIM1-expressing cells (hTIM1-293T) as well as cells naturally expressing TIM1 (Vero and A549 cells). This inhibitory effect was shown to be specific for TIM1-mediated entry of viruses through interaction of duramycin with PE present in the viral membrane (42). Through inhibition of Zika virus binding to TIM1, duramycin has also been shown to be effective in reducing infection of placental cells and explants (141).

Labyrinthopeptin A1 has shown promising antiviral activity against several viruses, including human immunodeficiency virus (HIV) and herpes simplex virus (HSV), with activity against laboratory-adapted strains and clinical isolates (including drug-resistant strains) (43). Labyrinthopeptin was able to inhibit cell-free viral infection as well as inhibit cell-to-cell spread of HIV *in vitro*. This inhibitory activity was dependent on time of drug administration and was only effective if the drug was administered within 1 h after infection. Like duramycin, these results suggest that labyrinthopeptin A1 likely also interferes with the viral entry process. Labyrinthopeptin A1 was shown to interact with the virus (interaction with envelope protein gp120) and not receptors on the host cell. However, binding to the virus is highly likely to be via interaction with lipids (specifically PE) in the viral membrane (44). A moderate degree of synergism when combined with other commonly used antiretroviral therapies was seen (43). An advantage of not having significant antibacterial activity is that labyrinthopeptin A1 does not have a negative effect on host microbiota, such as vaginal lactobacilli, reducing the risk of dysbiosis (43). Importantly, labyrinthopeptin A1 did not stimulate targeted immune cells (peripheral blood mononuclear cells [PBMCs]), as expression of CD69 and CD25 remained unchanged and did not result in significant induction of inflammatory cytokine secretion from these cells (43). Additionally, labyrinthopeptin A1 was not cytotoxic against vaginal epithelial cells or other nonepithelial cells at effective antiviral concentrations (43).

Labyrinthopeptin A1 and A2 have also been tested against a variety of other enveloped viruses, with labyrinthopeptin A1 being the most effective, conferring broad-spectrum antiviral activity (44). Of interest is the observation that labyrinthopeptins bind to PE and may be responsible for labyrinthopeptin binding to viral membranes. Furthermore, it was shown that the antiviral effect was a result of virolysis (viral membrane disruption), although similar effects on TIM1-mediated entry to those reported for duramycin cannot be excluded (42, 44). Additionally, labyrinthopeptins are effective against respiratory syncytial virus (RSV) *in vitro* and have shown promising results *in vivo* (45). Moreover, the labyrinthopeptins are not affected by resistance mutations usually detrimental toward RSV entry inhibitors. The mode of action against RSV is similar to that reported for other viruses (i.e., interaction with the virus-associated PE) (45). Although promising results were reported using an *in vivo* murine model, treatment was not as effective compared to *in vitro* models and requires additional research (45).

Efforts at generating lanthipeptides new to nature have also shown promise for generating lanthipeptides capable of inhibiting HIV budding from cells (142). A bacterial reverse two-hybrid (BRTH) system was used to screen potential lanthipeptide analogues for their ability to inhibit the interaction of the HIV p6 protein with the ubiquitin E2 variant (UEV) domain of human TSG101 (important for budding of HIV from infected cells). Using prochloricin A2.8 as a backbone, the amino acids between the two rings were randomized and modification performed by ProcM (LanM). *In vitro* testing using the BRTH system resulted in the identification of one peptide, XY3-3, capable of disrupting the interaction between HIV p6 and UEV. Further *in vitro* testing revealed that the lanthipeptide had more than 10-fold increase in activity compared to a previously

identified inhibitor and specifically binds to UEV (142, 143). Both lanthionine bridges were also shown to be crucial for activity. To access the peptides' ability to prevent Gag-mediated budding of virus-like particles in cell-based assays, the cell-penetrating Tat peptide was fused to the N terminus of XY3-3. The newly generated XY3-3-Tat was not toxic to cells at concentrations up to 500 nM and inhibited viral budding by 65% at 100 nM. The peptide interfered with the degradation of the epidermal growth factor receptor (at 500 nM), which is mediated by the UEV domain of TSG101, further supporting binding of XY3-3-Tat to UEV. Although further testing is required, lanthipeptides such as XY3-3-Tat and labyrinthopeptins may prove useful in antiviral therapy. Furthermore, methods based on BRTH and phage display systems provide a platform for identifying and testing novel lanthipeptides (57, 142).

HETEROLOGOUS EXPRESSION OF LANTHIPEPTIDES

Most studies investigating lanthipeptides require pure peptide at relativity high yields. This can be troublesome as some of the producing strains suffer from low production yields and production of other contaminating peptides. Furthermore, for a lanthipeptide to be commercially viable, industrial-scale production would need to be feasible. Additionally, lanthipeptides that are identified by genome mining are not always readily produced by the native producer or the native producer is not available (144). Furthermore, regulation of lanthipeptide and associated gene expression can be very complex and, in some cases, has not been fully elucidated. The design and expression of new-to-nature lanthipeptides are also not possible without the use of a heterologous expression system or chemical synthesis. Several heterologous expression systems have been investigated to produce lanthipeptides (Table 2). An important feature of any lanthipeptide expression system is the inclusion of the appropriate modification enzymes. In most cases, the precursor peptide is expressed to neutralize the bioactivity of the core peptide, reducing potential toxic effects of the core peptide against the expressing host. Furthermore, addition of affinity tags, such as multiple histidines, aids in purification via affinity chromatography.

As the native producer of nisin, it is not surprising that *Lactococcus lactis* has been used as a heterologous host to produce lantibiotics (Table 2). Additionally, the genes involved in the regulation of nisin expression have also been incorporated into a commercially available expression system, namely, the nisin-controlled gene expression system (NICE). In the native nisin-producing bacterium *L. lactis*, nisin biosynthesis is autoregulated by a two-component regulatory system made up of NisK (histidine sensor kinase) and NisR (transcriptional activator) (145). Mature nisin acts as its own inducing peptide, with NisK acting as its receptor. Once nisin is bound to NisK, a signal transduction cascade is initiated that results in the autophosphorylation of NisK. This subsequently results in the transfer of phosphate to NisR, which binds to the promoter regions in the nisin operon resulting in induced expression. This regulatory machinery is extremely sensitive and tightly controlled, which makes it ideal for use in a heterologous expression system. Using *L. lactis* as the heterologous host, yields of ~6.0 mg/liter (precursor peptide) have been reported (146). *L. lactis* peptides have been expressed from class I and II by using modifications of enzymes from both classes (147). A rapid screening system (nanoFlemming) using *L. lactis* has also been developed capable of assessing peptide libraries at nanoliter scale (58). Systems like these allow for the rapid screening of large peptide libraries that can significantly streamline the discovery pipeline.

Escherichia coli is a molecular workhorse, and a wealth of resources are available, ranging from different expression strains, cloning tools, and expression systems reviewed in reference 148. Due to these advantages, *E. coli* has been utilized as a heterologous expression host to produce lanthipeptides from all four classes (Table 2). From these studies, it seems that the multifunctional synthetases (LanM, LanKC, and LanL) are simpler to express in *E. coli* than the dedicated class I synthetases (i.e., LanB and LanC). This may be due to the increased complexity of modification, specifically the

requirement for two dedicated modification enzymes. Shi et al. (149) were the first to report successful expression of a class I lantibiotic in *E. coli* with *in vivo* modification of precursor nisin by LanB and LanC. Using this system, a yield of 24 mg/liter modified precursor nisin (13.8 mg/liter core peptide theoretical) was obtained. To further improve on this yield, the inclusion of optimized $tRNA^{Glu}$ sequences and glutamyl tRNA synthetase can increase the efficiency of class I modification enzymes (9). Shi et al. (149) also successfully expressed the class II lanthipeptides haloduracin (Halα and Halβ) and prochlorosins (1.7, 2.11, 3.2, and 3.3), modified using their respective LanM synthetases. Yields of the various precursor prochlorosins ranged from 10 to 35 mg/liter and fully modified haloduracin precursor peptides from 1 to 2 mg/liter (149). Previously, precursor nukacin ISK was produced in *E. coli* at 1.5 mg/liter, which is significantly less than prochlorosins but similar to haloduracin (150). This seems to indicate that the type of lanthipeptide as well as the modification enzymes used can influence production yields. Interestingly, prochlorosins were obtained from the soluble fraction when modified, but are mostly in the insoluble fraction when ProcM (LanM) is absent (149). Sequestration of heterologously expressed peptides/proteins to inclusion bodies is a potential limitation (151). To address this, fusion of precursor peptides to "solubility enhancers" has been investigated, including green fluorescent protein (GFP) and mannose-binding protein (MBP) (56, 151, 152). Using these larger fusion tags has been shown to improve stability and solubility of heterologously expressed proteins and can reduce toxicity to the heterologous host (56, 151–153). Importantly, fusion to these larger proteins does not interfere with the modification and may improve the contact time of the precursor peptides with their respective synthetases. An additional advantage of using GFP is its fluorescence, which can help in optimization of expression through real-time *in vivo* monitoring of expression and evaluation of purification (151–153). Although using these larger fusion proteins can result in reductions of final lanthipeptide yield, further optimization of these systems is required to unleash their full potential. Another possibility is to secrete the lanthipeptides outside the cell. This has been done successfully for the two-component lantibiotic lichenicidin, with yields of 4 and 6 mg/liter for the alpha- and beta-peptides, respectively (154). In this example, secretion and cleavage are performed by the bifunctional LanT (LicT), with additional cleavage performed by an extracellular protease LanP (LicP). This system has the advantage of not having to cleave the precursor peptides *in vitro* after purification, but removes the ability to utilize affinity chromatography during initial purification. Secretion can, however, still be performed without leader peptide cleavage, using *E. coli* secretion systems (e.g., the twin-arginine translocation [TAT] pathway) or by removing/disrupting the protease domain of the bifunctional LanT (if using a bifunctional LanT) (155).

Streptomyces spp. are known for their production of secondary metabolites and RiPPs, making them an intriguing platform for heterologous lanthipeptide production (156, 157). *Streptomyces* spp. have been used for the heterologous production of lanthipeptides represented in three of the four classes (II to IV), including cinnamycin, labyrinthopeptins, and streptocollin (124, 158, 159). Using *Streptomyces lividans*, fully modified labyrinthopeptides could be produced at yields of 86 and 14 mg/liter for labyrinthopeptin A1 and A2, respectively (158). These values are lower than those produced by the wild type (90 and 36 mg/liter for A1 and A2, respectively), but it should be noted that this process was not optimized, and more importantly, copurification of closely related peptides is eliminated when the peptides are heterologously expressed (43, 158). Similarly, actagardine was also successfully expressed in *S. lividans*, with yields of 50 to 80 mg/liter (159). Expression of streptocollin in *Streptomyces coelicolor* also proved fruitful, yielding a 5.5-fold increase in production over the wild-type strain (from 1.8 mg/liter to 10 mg/liter) (124).

In addition to the use of these expression systems to produce lanthipeptides, they can also be used for fundamental studies and as tools in the drug discovery pipeline. To this end, heterologous expression has been used in several studies to investigate

fundamentals of lanthipeptides, including structure-function relationships, gene function, and regulation of expression. This is, in part, made possible by genetic tools available for heterologous hosts, such as *E. coli* and *L. lactis*, which allow for specific manipulation of genes involved in the modification and processing of the peptides or to easily make changes to peptide structure and study the effect on bioactivity. The refactoring of promoters and transcriptional units for target gene clusters can be used to improve expression in heterologous hosts and facilitate the expression of pathways that are silent in the producing strain. Furthermore, rapid screening methods have also been developed and are essential for testing of large libraries of potential lanthipeptides (56–58).

CONCLUDING REMARKS

Lantibiotics (and lanthipeptides in general) represent a diverse range of peptides and make up the largest group of RiPPs. Due to this diversity, a plethora of bioactive peptides have been discovered, with activities as diverse as the peptides themselves. Recent studies illustrating the widespread nature of lanthipeptides in bacterial genomes are promising, signifying an untapped source of potential biotherapeutics with novel mechanisms of action. This is especially important in current times, given the rise in resistance toward available therapeutic interventions. It is therefore promising to see the significant advances in the lantibiotic/lanthipeptide discovery pipeline over the past few years. These include methods for rapid evaluation of large lanthipeptide libraries and development of more effective production systems.

However, there remains an innovation chasm between academic research and commercialization of lanthipeptides. To help bridge this gap, future studies should focus on identifying potential applications of novel peptides and evaluating their modes of action. Additionally, increased focus should be placed on *in vivo* assessment to help identify and address shortcomings, such as low bioavailability. Another aspect that requires further innovation is the production of lanthipeptides. Despite significant steps being made in the heterologous expression of lanthipeptides, the complex nature of their PTMs and low production yields remain a hurdle. Future research needs to focus on the fine-tuning of expression systems to produce lanthipeptides at feasible yields and see lanthipeptides enter the commercial market.

SUPPLEMENTAL MATERIAL

Supplemental material is available online only.

SUPPLEMENTAL FILE 1, PDF file, 0.9 MB.

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