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Review

Non-lipid II targeting lantibiotics

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1. Lantibiotics

In the microbial world the continuous struggle for survival has led to the evolution of secreted peptides, proteins and other compounds that are able to inhibit the growth of or even kill competitors. These peptides and proteins are, by virtue of their activity, called bacteriocins. The first bacteriocin, Colicin V, was found in 1925 and ever since bacteriocins have been continuously discovered [1,2]. The first lantibiotic, nisin, was discovered in 1928 [3] and much later, in 1971, its structure was determined (Fig. 1) [4]. Many more lantibiotics have been discovered since and they have their own class within the bacteriocins (Class I) [5]. The most distinguishing features of the lantibiotics are the thioether-linked rings and the dehydrated amino acids, which are introduced in the peptides post-translationally. The typical thioether linkages, provided by the lanthionine (Lan) or β-methyllanthionine (MeLan) have first been found in wool, which is why this family of peptides has been called lantibiotics, derived from the Latin word *lana* (wool) [6]. Lantibiotics are ribosomally synthesized and mostly produced by gram-positive bacteria [6,7]. Their post-translational modifications are essential for antimicrobial activity. Lantibiotics are active against antibiotic-resistant bacteria such as methicillin-resistant *Staphylococcus aureus* (MRSA) and vancomycin-resistant enterococci (VRE), thus they are considered promising candidates to deal with the antibiotic-resistance problem. Furthermore, lantibiotics have great potential for application as food additives and veterinary medicine as they are active against food borne pathogens such as *Listeria monocytogenes*, *Salmonella typhimurium* and *S. aureus*. Nisin, the most well studied lantibiotic, is the only one commercialized in pure form as food preservative and in animal care products to date [8]. The elucidation of the mode of action of nisin has taught us that the low minimum inhibitory concentration (MIC) in the nanomolar range of lantibiotics against pathogens often involves a specific target [9], to which the lantibiotics bind with high affinity. Studying the antimicrobial mechanisms of lantibiotics helps us to understand how they kill, and can possibly provide us with novel ways to tackle important pathogens.

Among the lantibiotics, compounds have been found that do not possess antimicrobial activity but serve other purposes [10–12]. Hence the term lanthipeptides was coined for these type of peptides [13]. Lantibiotics are sub-divided into a type-A and a type-B group. Type-A lantibiotics are positively charged peptides with an elongated shape whereas type-B lantibiotics are neutral or negatively charged peptides with a more globular shape [6]. Besides these differences in charge and shape, type-A and type-B lantibiotics are differently post-translationally modified. In principle, these modifications performed by a series of enzymes, are necessary to obtain full antibiotic activity. The lantibiotic biosynthetic genes are situated in a single gene cluster that is responsible for the lantibiotics' production, modification, export, regulation of expression and self-immunity. The precursor peptides of lantibiotics referred to as pre-peptides are encoded by the *lanA* genes [14]. These precursors contain a leader peptide at the N-terminus, rendering them biologically inactive [6]. The pre-peptides need to be modified by dehydration and cyclization to introduce the dehydrated amino acids and the Lan/MeLan rings. These modifications are performed by the dehydratase LanB and the cyclase Lan C for the type-A lantibiotics, and by the bi-functional enzyme LanM (for both activities) for the type B lantibiotics [15]. Proteases that remove the leader peptide of the lantibiotics and the proteins responsible for the secretion also differ

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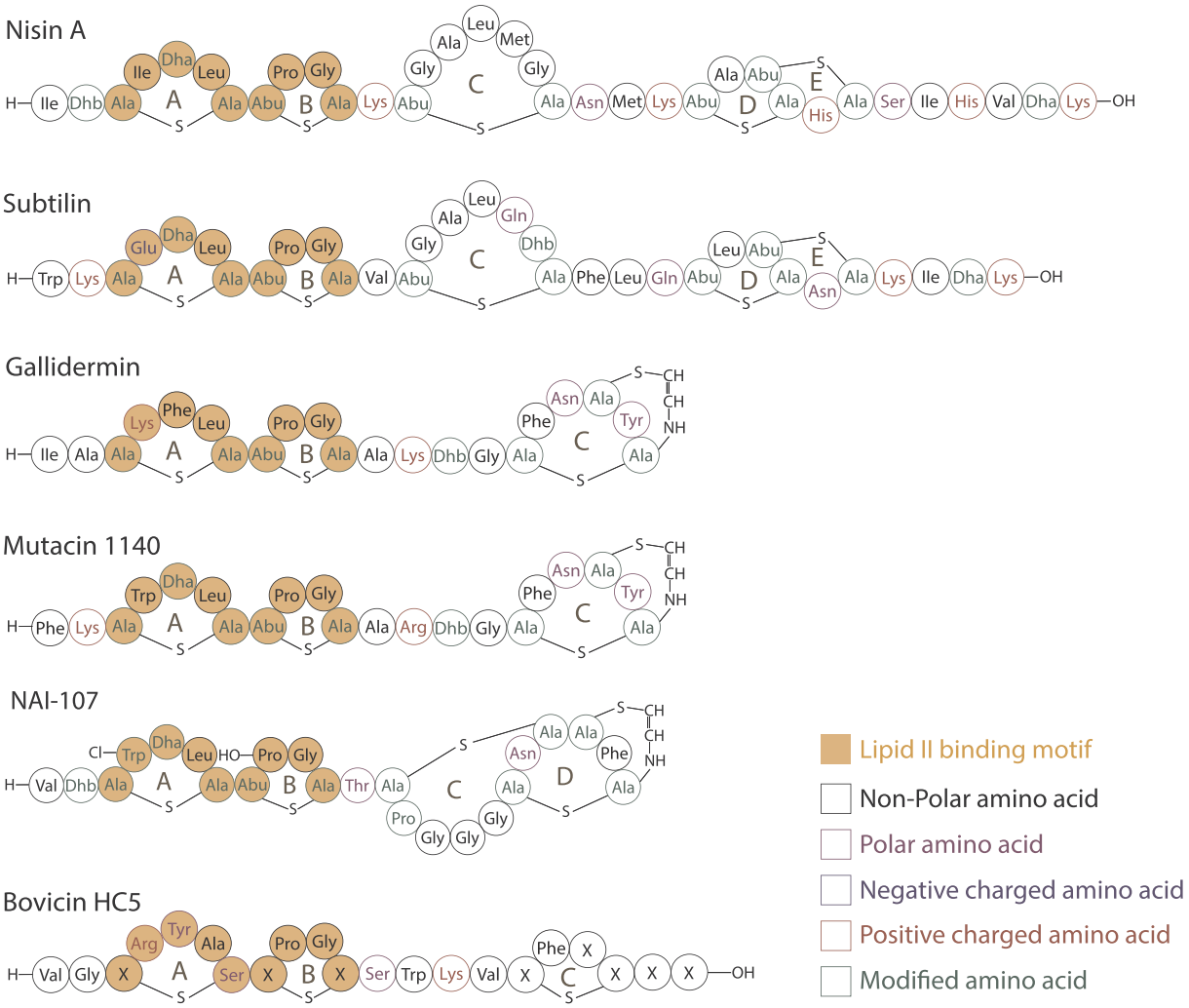

Fig. 1. Lantibiotics of the nisin group that bind to Lipid II. Structures of the lantibiotics nisin A, subtilin, gallidermin, mutacin 1140, NAI-107 and bovicin HC5. NAI-107 has two congeners namely HO-Pro14 and di HO-Pro14. Some of the amino acids in bovicin HC5 are not identified yet, and are marked with an X. The lipid II binding motif is highlighted in orange.

between the type-A and type-B lantibiotics. For type-A lantibiotics, the ATP-binding cassette (ABC) transport protein LanT secretes leader peptide attached lantibiotics, which is followed by cleavage of the leader peptide extracellularly by the serine protease LanP [16,17]. For type-B lantibiotics, proteolysis and secretion are mediated by the bi-functional enzyme LanP, which is a transport protein containing a proteolytic domain [15]. The gene encoding the protease is sometimes found outside the *lan*-gene cluster. For instance, there is no protease gene in the gene cluster of the strain producing subtilin, although its leader peptide is removed, suggesting that the leader peptide of this lantibiotic is cleaved by another protease [18]. Removal of the leader can also be done by a protease before the lantibiotics are secreted (e.g., cinnamycin) [16,19]. In addition to the genes involved in processing, regulatory genes and self-immunity genes are important for the production of lantibiotics. The two-component regulation system LanKR is often found in both type-A and type-B producing strains [20]. The histidine kinase LanK functions as a receptor and the transcriptional regulator LanR works as a response regulator for the production of lantibiotics [21]. The products of the three genes *lanEFG* together form a ABC-like transporter that is responsible for active extrusion of the lantibiotics from the membrane, thus keeping the concentration at the membrane of the lantibiotic below a certain threshold [22]. Additionally, bacteria producing lantibiotics such as pep5, cytolysin and lactocin S require protection by LanI [6,23–26]. The LanI protein is present mostly at the external leaflet of the plasma membrane where it is anchored to the membrane either by a lipid anchor (e.g. for SpaI and NisI) or via one or more transmembrane segments (e.g. PepI and LtnI). There are several mechanisms postulated how the LanI proteins protect the producing cell from the action of the lantibiotic [27,28]. For instance, NisI, the immunity lipoprotein of nisin, has been shown to bind to nisin thereby preventing nisin from killing the cell [27]. The immunity mechanism of PepI, the immunity protein of pep5, is still unknown. The potential target of pep5 is assumed to be on the membrane, and is likely an anionic compound considering the highly positively charged nature of pep5. Pepl also contains 8 positive charged amino acids in a C-terminal domain of 20 amino acids, hence it was postulated that PepI protects the producing cell from pep5 by preventing the interaction with its target [28].

2. Two types of lantibiotic-Lipid II binding motifs

The most common target for lantibiotics is the polyisoprenoid-linked cell wall precursor Lipid II [9]. Two different Lipid II-binding modes can be distinguished within the lantibiotics family, namely the pyrophosphate cage formed by the A and B-ring system of nisin and related lantibiotics (Fig. 1) and the mersacidin type of binding mode that is also present in the lacticin 481 group and the two-component lantibiotics lacticin 3147 and haloduricin (Fig. 2). Nisin, the most well-

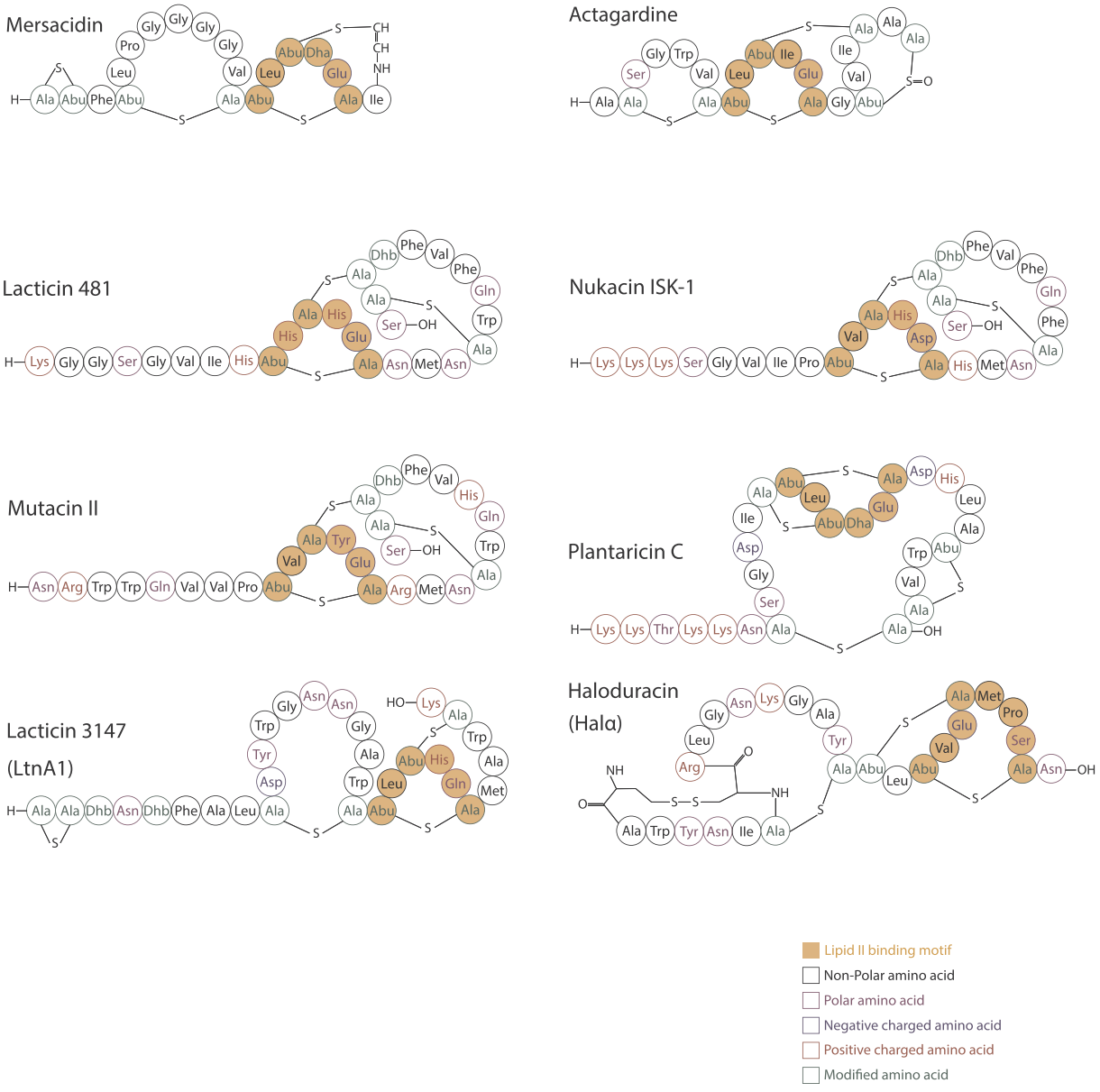

Fig. 2. Lantibiotics that bind to Lipid II of the mersacidin group. Structures of the lantibiotics mersacidin group (mersacidin, actagardine), lacticin 481 group (lacticin 481, nukacin ISK-1, mutacin II), plantaricin C, LtnA1 of lacticin 3147, Halα of haloduracin. The lipid II binding motif is highlighted in orange.

known lantibiotic, uses Lipid II as an anchor molecule to form pores in the target cell's membrane [29–31]. The A and B rings of Nisin form a pyrophosphate cage and bind to the pyrophosphate moiety of Lipid II forming nisin-Lipid II complexes. The D and E rings of nisin are important for insertion and pore-formation in the membrane leading to cell death [29,32,33]. So far, all the lantibiotics that have similar A/B-ring systems, like Mutacin 1140, gallidermin, epidermin, subtilin, NAI-107 and bovicin HC5, have been shown to target Lipid II (Fig. 1) [34–38].

Lantibiotics from the mersacidin-group bind to Lipid II differently. Mersacidin was shown to also require the GlcNAc for binding and thus binds the complete headgroup of Lipid II [39], pp-MurNAc(pentapeptide)-GlcNAc, whereas for nisin Lipid I (lacking the GlcNAc sugar) is sufficient [40]. The lantibiotics from this group do not induce membrane leakage but rather inhibit cell wall synthesis by binding to Lipid II [41]. Notable is the presence of a negatively charged glutamate or aspartate in the conserved motif with which these lantibiotics bind to Lipid II (Fig. 2). The presence of this glutamate was shown to be essential as mersacidin was inactivated when Glu17 was replaced with Ala17 [42]. The essential role of this glutamate explains the requirement for Ca^{2+} for maximal activity, where a calcium ion is likely necessary for bridging the negatively charged pyrophosphate group of Lipid II to the negative charge of the glutamate side chain [39,43].

An interesting combination of the two ways lantibiotics use Lipid II to kill bacteria is found in the two-peptide lantibiotics lacticin 3147 (LtnA1 and LtnA2) and haloduracin (Fig. 3) [44,45]. The LtnA1 and Halα peptides contain a mersacidin-like Lipid II binding motif and thus bind to Lipid II. However, this interaction does not lead to permeabilization of the target membrane. Only in the presence of the partner peptides (LtnA2 or Halβ) permeabilization of the membrane (presumably by pore-formation) is achieved [45].

In addition to lantibiotics that bind to Lipid II, there are lantibiotics that have quite different modes of action. Some of these lantibiotics kill bacteria even more efficient than nisin and their antimicrobial mechanisms do not involve Lipid II, as will be discussed below.

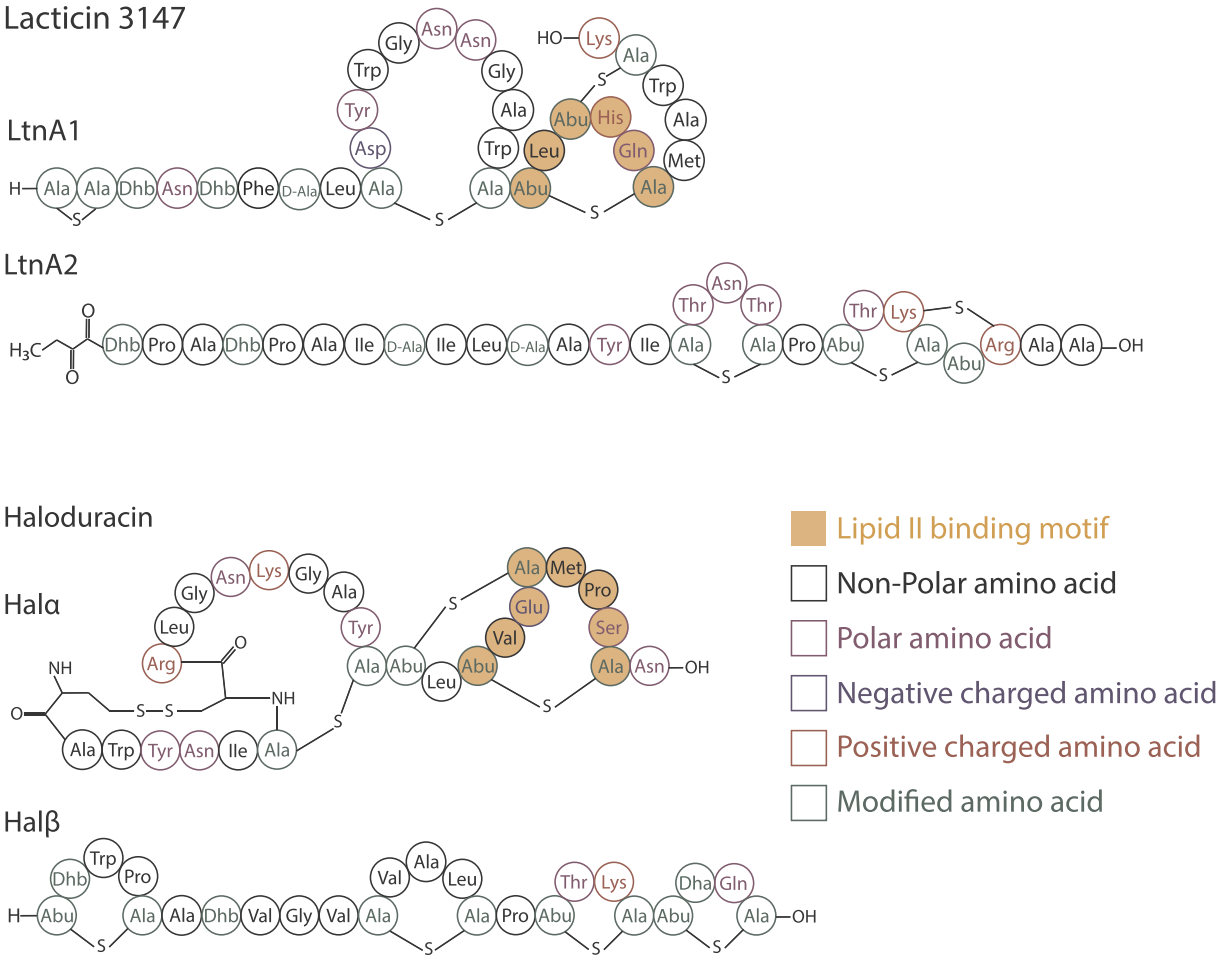

Fig. 3. Two-peptide lantibiotics. Structures of the lacticin 3147 (LtnA1 and LtnA2), haloduracin (Halα and Halβ). The lipid II binding motif is highlighted in orange.

3. Cinnamycin group lantibiotics (cinnamycin, duramycin, duramycin B, duramycin C and ancovenin)

Lantibiotics from the cinnamycin group are probably better known for their property of specifically binding to the phospholipid phosphatidylethanolamine (PE) than for their antimicrobial abilities. Cinnamycin, also named Ro 09–0198, was first isolated in 1952 and later others were found with a similar structure [46–49]. These lantibiotics are mainly produced by *Streptomycetes* spp. and contain 19 amino acids, one Lan and two MeLan residues (Fig. 4) [48]. Cinnamycin, duramycin, duramycin B, and duramycin C have a special lysinoalanine (Lal) bridge between Ala6 and Lys19 and an erythro-3-hydroxy-L-aspartic acid at position 15 which is important for their antimicrobial activity [50]. This lysinoalanine bridge was not reported for ancovenin [51], but given the high structural resemblance of the peptides in this group it is likely that this bridge is present also in this peptide. (See Fig. 4).

Duramycin displays MICs in the range of 2–3.75 μM against *Bacillus spp.*, 1.7 μM against *Paenibacillus BC26*, and it acts against *Herbspifillum* YR522, *Variovorax* CF313, *Chryseobacterium* CF314 G, *Sphingobium* AP49 with MICs of 17.5, 20, 21, 32.5 μM, respectively [52]. It also shows broad range activity against gram-negative bacteria, which may relate to its antimicrobial mechanism since PE is commonly present in relatively high amounts in both the outer and the plasma membrane of gram-negative bacteria.

The antimicrobial mechanism of the cinnamycin group is related to their ability to bind to PE at a 1:1 stoichiometry with high affinity [50,53]. This property is also likely the cause of the interest for these peptides in fields unrelated to infectious diseases. Duramycin forms ion channels in both artificial and biological membranes and was reported to enhance chloride secretion in airway epithelium [54,55]. Thanks to the latter property, it has entered clinical trials to relieve the burden of cystic fibrosis in patients [56].

Ancovenin was actually discovered in a screen that was set up to identify inhibitors of the angiotensin I converting enzyme which is a membrane-bound peptidyl dipeptidase [49,57]. This may be related to the ancovenin-PE binding as well. Duramycins also show broad-spectrum inhibition of viral entry into mammalian cell [58]. Because of the PE-specific targeting of the peptides from the cinnamycin group, they have been labeled fluorescently or with other tracers and employed to study the localization of PE in eukaryotic membranes [59]. In view of their overall performance in the above described areas it's likely that the application of this group will eventually be restricted to PE-localization studies.

4. Lactocin S

Lactocin S (3761.9 Da) produced by *Lactobacillus sakei* L45, was first isolated in 1989, and its amino acid sequence was determined in 1991 [60,61]. It is encoded on the 50 kb plasmid pCIM1, which is unstable and easily lost during bacterial reproduction [62,63]. We had the same experience in our attempts to purify this lantibiotic (unpublished observations). Lactocin S is a lantibiotic of 36 amino acids with a relatively low amount of modifications with a lactate group blocking the N-terminus and containing the typical lantibiotic-related residues (Fig. 5). Notably, the peptide contains three D-Ala residues, which are post-

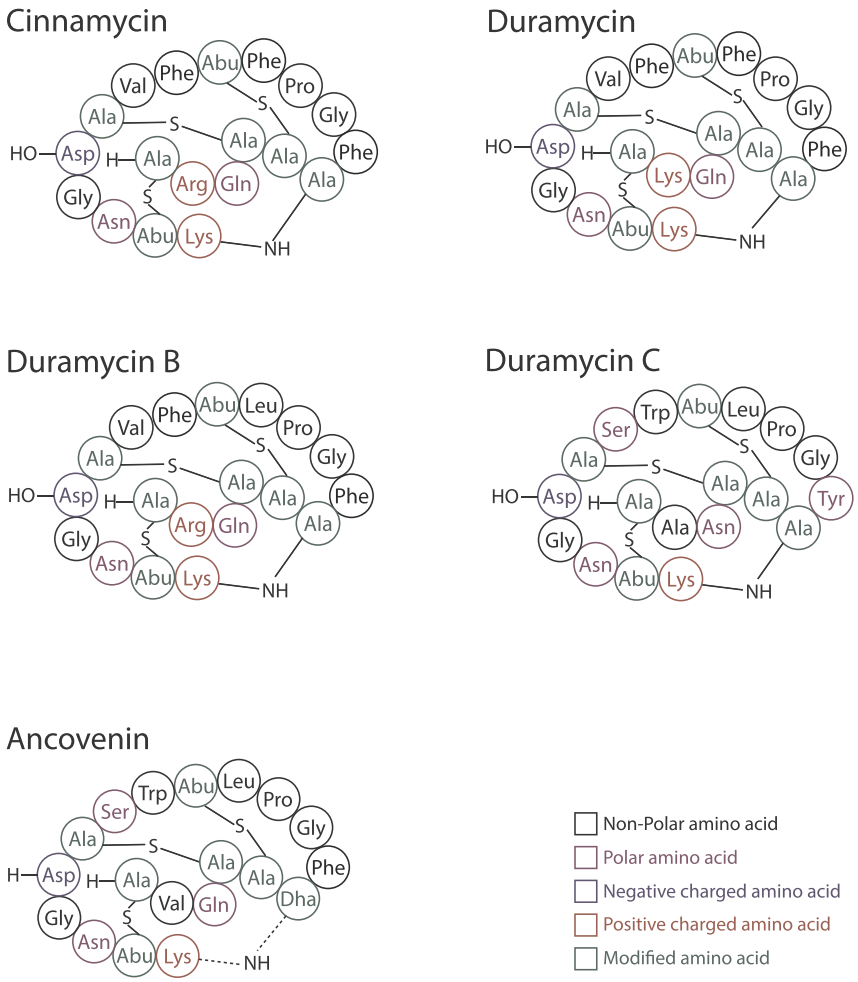

Fig. 4. Cinnamycin group lantibiotics. Structures of the lantibiotics cinnamycin, duramycin, duramycin B, duramycin C and ancovenin.

translationally converted from L-serines of the pre-peptide [64]. Lactocin S contains 2 positively and 2 negatively charged amino acids. Additionally, it contains 2 histidine residues at ring B, which will render the peptide positively charged at pH conditions below the pKa of histidine (6.0) [65]. So far no homolog of lactocin S has been identified or characterized, making this a unique molecule within the lantibiotics family.

Lactocin S displays MICs in the range of 0.038–0.36 μg/ml against lactic acid bacteria (LAB), 0.39–1.2 μg/ml against *Staphylococcus spp.*, 0.49–0.74 μg/ml against *Listeria spp.*, 0.43 μg/ml against *E. faecalis* EF, and 2.0 μg/ml against *B. cereus* [66]. Furthermore, lactocin S inhibits a broad range of food-borne pathogens. *Lactobacillus sakei* L45 is generally considered as safe for food products and is also used as a probiotic. Lantibiotic-producing lactic acid bacteria such as L. *sakei* L45 have probably their highest potential in food fermentation and preservation.

Likely due to the presence of the histidines, lactocin S only shows antimicrobial activity at pH values below 6.0, pointing to the importance of a positively charged C-terminal ring-system. Lactocin S and derived analogues have been fully synthesized chemically by the group of Vederas [61]. This allowed the first structure-activity study of this lantibiotic that showed that the antimicrobial activity of lactocin S was enhanced when the thioether bond of ring A was replaced by a methylene [67]. However, a similar replacement in ring B significantly reduced the activity [67]. These results together with the pH dependency of the activity point to an important role of ring B in the mode of action of this peptide. The low MIC towards LABs indicates that lactocin S uses a specific target, which remains to be identified. Considering the bactericidal activity of lactocin S below pH 6, it is likely that the peptide is membrane active.

5. Paenibacillin, subtilomycin, thuricin A4-4

The paenibacillin group is the lantibiotics group with the most post-translational modifications per peptide (Fig. 6). Paenibacillin (*m/z*, 2984.6) is produced by *Paenibacillus polymyxa* OSY-DF and was structurally characterized in 2008 [68,69]. Subtilomycin (*m/z*, 3235.6) and thuricin A4-4 (*m/z*, 2786.3), produced by *Bacillus* spp. were structurally characterized in 2013 and 2015, respectively [70,71]. Paenibacillin has 30 amino acids and its first N-terminal amino acid is acetylated which is uncommon for lantibiotics [69]. Subtilomycin and thuricin A4-4 consist of 31 and 27 amino acids, respectively, and their N-terminal amino acids are modified with 2-oxo butyrate (Obu) [70,71]. The structure of the B and C rings of the peptides in this group are similar to the D and E rings of nisin.

Paenibacillin displays MICs in the range of < 0.2–1.56 μM against MRSA, 0.1–0.78 μM against *E. faecalis* including VRE, and in the micromolar range against pathogenic gram-negative bacteria including those of the ESKAPE-group, i.e. six bacterial pathogens (***E**nterococcus faecium*, ***S**taphylococcus aureus*, ***K**lebsiella pneumoniae*, ***A**cinetobacter baumannii*, ***P**seudomonas aeruginosa*, ***E**nterobacter spp.*) that are highly related to antimicrobial resistance [72]. Subtilomycin exhibits activity against a broad spectrum of gram-positive bacteria such as *B. cereus*, *L. monocytogenes* and *Clostridium sporogenes* [70]. It also inhibits a few gram-negative bacteria but these are less sensitive to subtilomycin compared to the gram-positive bacteria [70]. Thuricin 4A-4 display

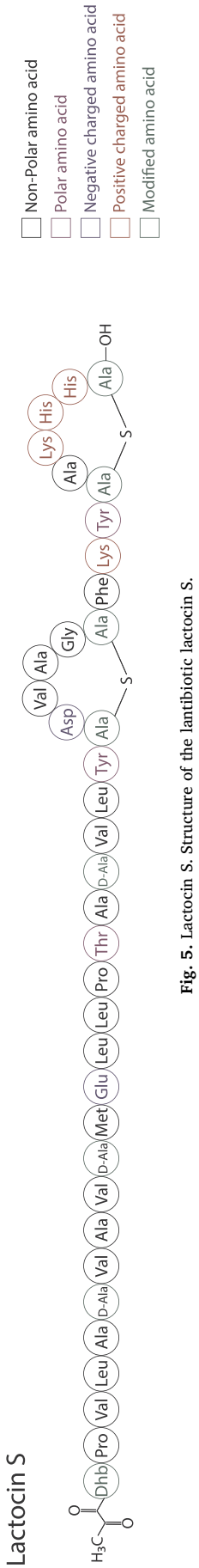

Fig. 5. Lactocin S. Structure of the lantibiotic lactocin S.

MICs in the range of 0.326–7.8 μM *against Bacillus* spp., 1.3–1.95 μM against *Staphylococcus* spp., but it is inactive against gram-negative bacteria [71].

Paenibacillin shows higher ability to penetrate the outer membrane of bacteria as compared to nisin at concentrations up to 60 μM, while it is not haemolytic nor cytotoxic to mammalian cells [72]. These properties led the authors to have high hopes for the paenibacillins "as a scaffold for further development as a next-generation antibacterial drug to fight difficult-to-treat pathogens", however protease-sensitivity due to the presence of 5 lysines will likely prevent this. Very recently, subtilomycin was shown to act as a masking agent to reduce the defense of *Arabidopsis thaliana* and in effect promote colonization by the endophyte [73]. Whether this is a general property of this lantibiotic group is unknown, but the producing bacteria (*P. polymyxa* and *B. subtilis*) are both endophytes. This raises a question about the main purpose of these peptides; antimicrobial activity or masking from host defense mechanisms.

6. Pep5 and epicidin 280

Pep5 (3487.8 ± 0.3 Da) and epicidin 280 (actually two compounds with MW 3133 ± 1.5 Da and 3136.0 ± 1.5 Da) produced by *S. epidermidis*, were discovered in 1989 and 1998, respectively [74–76]. Pep5 and epicidin 280 consist of 33 and 29 amino acids, respectively (Fig. 7). They carry three Lan/MeLan rings, and the spacer between ring A and the rings B and C of epicidin 280 is 3 amino acid residues shorter compared to that of pep5. The N-termini of both peptides are blocked, by an Obu moiety in pep5 and a lactate group in epicidin 280. The 8 positively charged amino acids of pep5 render it the most highly charged peptide of the lantibiotic family. Epicidin 280 exhibits 75% sequence similarity to pep5 and their biosynthetic genes share significant homology as well [77,78]. This high similarity is probably the reason for the cross-immunity of their producing strains towards each other's peptide.

Pep5 has a highly selective antibacterial activity and displays MICs in the range of 0.001–19 μg/ml against *Staphylococcus* spp. and 1.2 μg/ml against *Micrococcus luteus*. The most active epicidin 280 compound displays higher MICs in the range of 0.175–3.85 μg/ml against *Staphylococcus* spp. and 9.7 μg/ml against *M. luteus* [77]. Especially the low nanomolar MICs against *Staphylococci* make pep5 a highly interesting lantibiotic, as apparently it uses a highly specific target.

Earlier research on the antimicrobial mechanism of pep5 showed that it causes membrane depolarization presumably via pore-formation [79,80]. However, compared to the MIC values of pep5 against staphylococci and micrococci, the concentrations of pep5 causing membrane depolarization were magnitudes higher. This difference in concentrations suggests that pore-formation may not be the prime antimicrobial mechanism of pep5. High concentrations of cationic antimicrobial peptides tend to form pores in membrane as a barrel-stave, carpet or toroidal-pore, which depends more or less on their amphipathicity and hydrophobicity [81,82]. In our hands, pep5 had no effect on the membrane potential at 50× the MIC and is bacteriostatic (unpublished). This is in line with previous research showing that trypsin can rescue the growth of bacteria that were pre-treated with pep5, even after several hours [74]. The lack of signature Lipid II-binding domains in the sequence of pep5 argues against Lipid II as its target. Accordingly, pep5 did not cause leakage from Lipid II containing vesicles in vitro [30]. Moreover, pep5 does not interact with Lipid II in vitro (despite its high number of positive charges) as addition of Lipid II did not antagonize pep5 in an agar diffusion assay, whereas a clear inhibitory effect on nisin was observed (Fig. 8).

Several pep5 structure-function studies have been performed, which showed that replacing the dehydrated amino acids at position 16 or 20 with an alanine already increased the MICs of very sensitive strains by a factor of 60–90. Incorporation of an extra lanthionine ring in the middle of the peptide abolished the activity of pep5 [83]. In our experience,

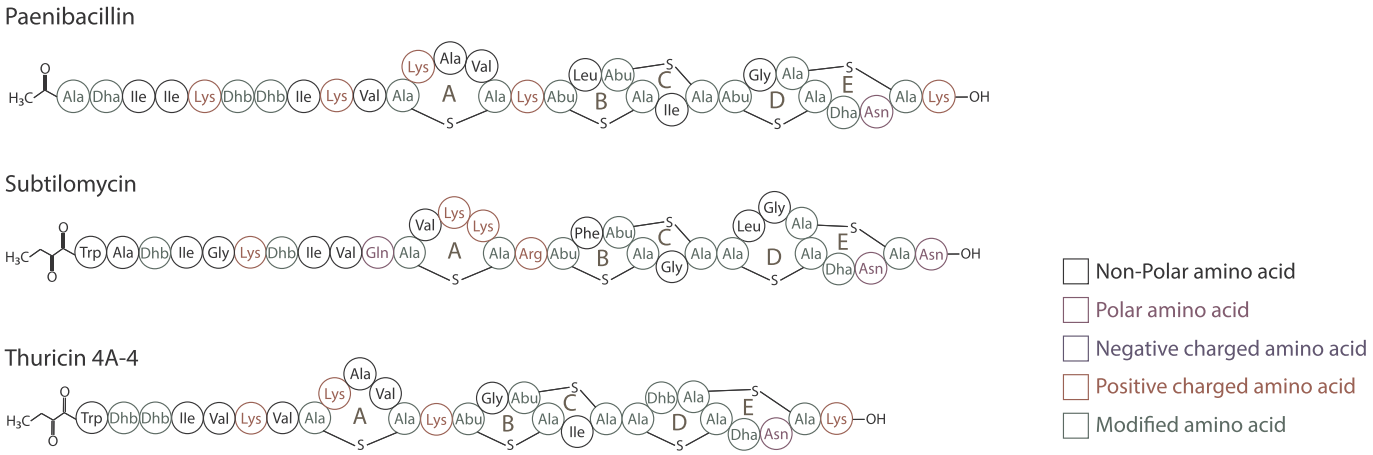

Fig. 6. Paenibacillin group of lantibiotics. Structures of the lantibiotics paenibacillin, subtilomycin, thuricin 4A-4.

pep5 is very sensitive to chemical modifications. Specific labeling of the N-terminus (via hydrazine-based coupling) or mild labeling using NHS-probes destroyed all activity of pep5 (S.F. Oppedijk and E. Breukink, unpublished observations). These properties blocked our attempts to analyze the binding of the peptide to intact cells. There is no information on the antimicrobial mechanism of epicidin 280, thus we cannot compare the modes of action.

7. Epilancin group lantibiotics (epilancin 15× epilancin K7 and SWLP1)

Epilancin K7 (3032 ± 1.5 Da), epilancin 15× (3173 Da) and SWLP1 (2999 Da), the main members of this group, are produced by *Staphylococcus* spp. and were discovered in 1995, 2005 and 2009, respectively [84–87]. They share extremely high structural similarity (Fig. 9). Epilancins have a lactate group that blocks the N-terminus of which the specific function remains unknown. Replacement of the lactate with a pyruvate in epilancin 15× resulted in a very minor decrease in antimicrobial activity, which suggest that the nature of this group is not important for the activity. Its function is likely to protect the peptides from degradation by proteases [88,89]. Besides a lanthionine (Ala12-S-Ala16) they contain a ring B and ring C system that is very similar to the D and E rings of nisin. The peptides have a high number of positively charge amino acids.

Epilancin 15× displays MICs in the range of < 0.125–0.5 μg/ml against VRE and MRSA, 12.5–50 μg/ml against Acinetobacter spp., 0.25–100 μg/ml against *Escherichia. coli* spp., 0.25–1 μg/ml against *E. faecalis* spp., < 0.125–1 μg/ml against *S. epidermidis* spp. [90]. SWLP1 displays MICs in the range of 32–64 μg/ml against VRE spp., 1.5 μg/ml against vancomycin-resistant *S. aureus* spp.(VRSA), < 1–8 μg/ml against *S. epidermidis* spp., 96 μg/ml against *Klebsiella pneumoniae* [87].

In our hands it turned out that it was essential to remove residual trifluoroacetic acid (TFA) from the preparations after the final HPLC step in order to have maximal peptide activity (X. Wang and E. Breukink, unpublished observations). This may be required for all highly charged lantibiotics that are purified by HPLC using TFA-containing mobile phases.

The epilancins are active towards VRE implying that they target a different binding site than vancomycin. For nisin, the C-terminal part including the rings D and E has been suggested to be important for pore-formation [29]. Hence, the similar structure of the epilancins might be involved in pore-formation as well. This notion is in agreement with the disruption of the membrane potential of intact bacterial cells at relatively low peptide concentrations shown for an epilancin 15× variant [88,91]. Notably, as is the case for pep5, the epilancins lack a signature Lipid II-binding element in their primary structure. Thus, it is reasonable to assume that the epilancin group lantibiotics do not target Lipid II. Indeed, it was shown for epilancin K7 that it could not induce leakage of contents from model membrane vesicles containing Lipid II [88,91]. In our studies on the mode of action of epilancin 15×, we surprisingly observed that Lipid II can antagonize the epilancin 15× activity in an agar diffusion assay (Fig. 10) although a higher concentration of Lipid II was needed to inactivate epilancin 15× as compared to nisin. Moreover, dioleoyl phosphatidyl glycerol (DOPG), the negatively charged lipid control, antagonized epilancin 15× also to some extent. This is likely due to the electrostatic interactions of the highly charged peptide with the negatively charged PG, suggesting that an initial electrostatic interaction of the peptide with the target bacteria or their membrane may be important. Epilancin 15× and Lipid II showed specific binding in vitro, and we could reproduce the earlier data from the Sahl group that this interaction did not lead to the formation of pores in model membranes (X. Wang and E. Breukink

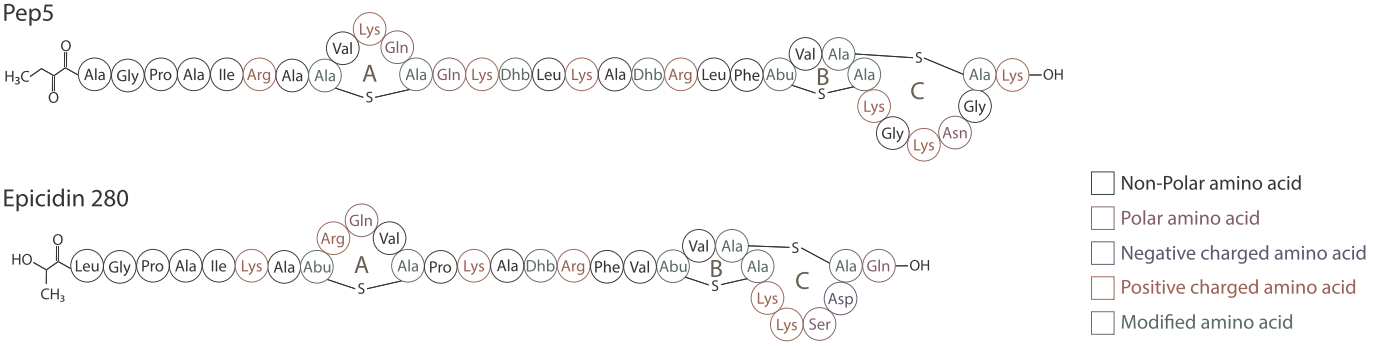

Fig. 7. Pep5 group of lantibiotics. Structures of the lantibiotics pep5 and epicidin 280.

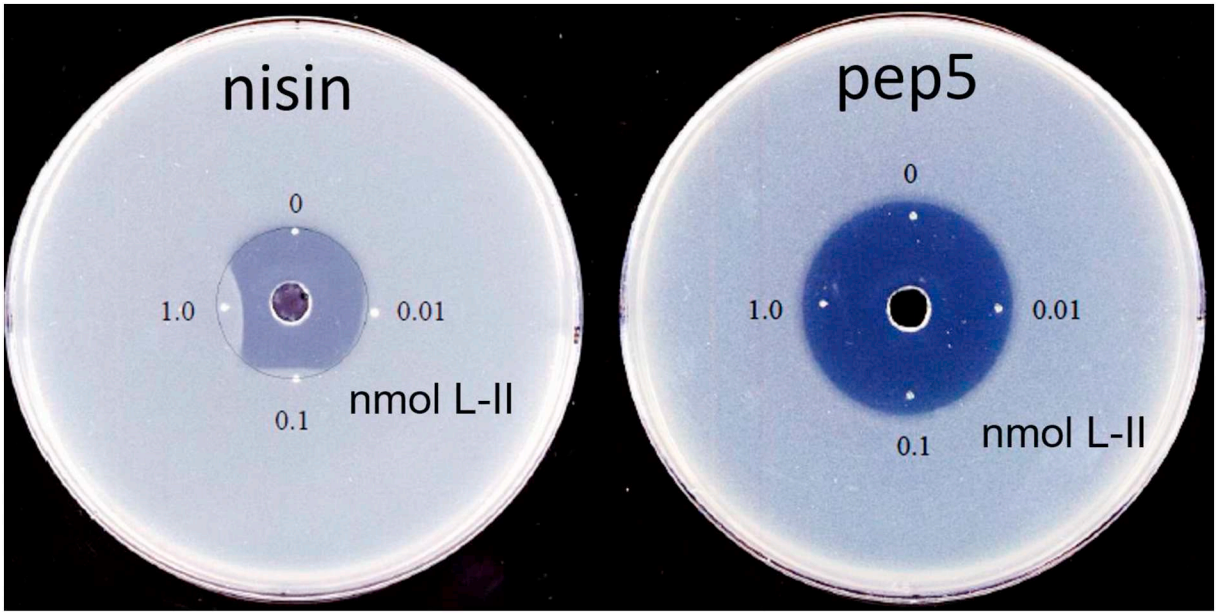

Fig. 8. Test for antagonization of pep5 activity by Lipid II. Pep5 and nisin are placed on the agar which is pre-mixed with the indicator strain *Staphylococcus simulans*. Different amounts of Lipid II (0.01–1 nmol) dissolved in 10 mM Tris-HCl pH 7.5, 100 mM NaCl, 0.1% Triton X-100, are spotted on the edge of the (predicted) inhibition halo and incubated overnight at 30 °C to allow growth of the indicator strain. The presence of Triton X-100 is to ensure that Lipid II is dissolved properly.

unpublished observations).

8. Outlook

This review mainly focused on non-Lipid II targeted lantibiotics of which the antimicrobial mechanisms are not clear. The majority of the lantibiotics bind to Lipid II, which is not so surprising since it is a very good and accessible target that does not easily acquire resistance via mutations changing its structure because it is not gene-encoded. However, resistance that occurs by secreting an enzyme that specifically cleaves nisin has been described [7,92–94]. We would not be surprised to see more strains obtaining a similar resistance mechanism against other Lipid II-binding lantibiotics, once they are used in the clinic.

Lantibiotics are easily degraded by proteases especially due to the abundant presence of positively charged amino acids, which make them non-ideal candidates for pharmaceutical applications. Yet, there are always exceptions to the rule, e.g. the Lipid II-targeting NAI-107 does not possess any lysine or arginine and was being developed for clinical use by Naicons (current status unknown) [95].

We noticed an interesting structural homology between the lantibiotics groups of which the targets have not been identified yet. Epilancin, pep5 and paenibacillin all have a ring composed of five amino acids (including the lanthionine residue) containing at least one positive charge. This ring is located approximately in the middle of the peptides. Two amino acids upstream of this ring a lysine is present in

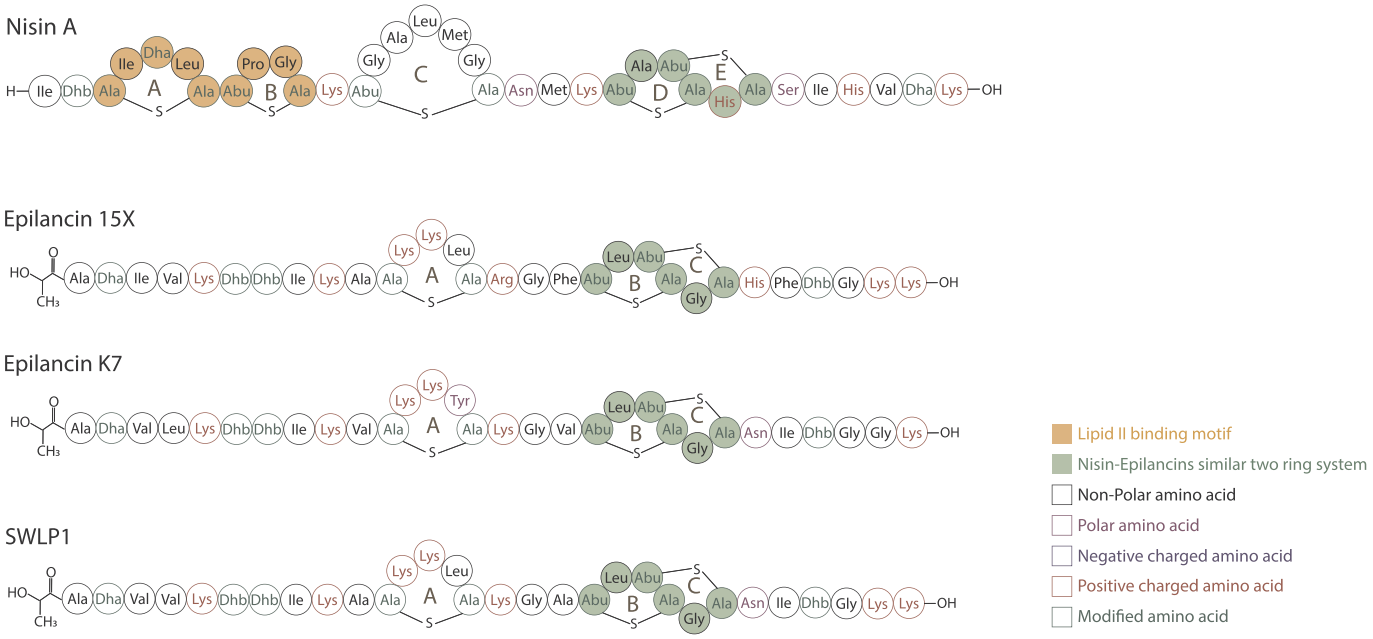

Fig. 9. Nisin A and lantibiotics of the epilancin group. Structures of the lantibiotics nisin A, epilancin 15X, epilancin K7 and SWLP1. The lipid II binding motif is highlighted in orange The similar two C-terminal ring systems are highlighted in green.

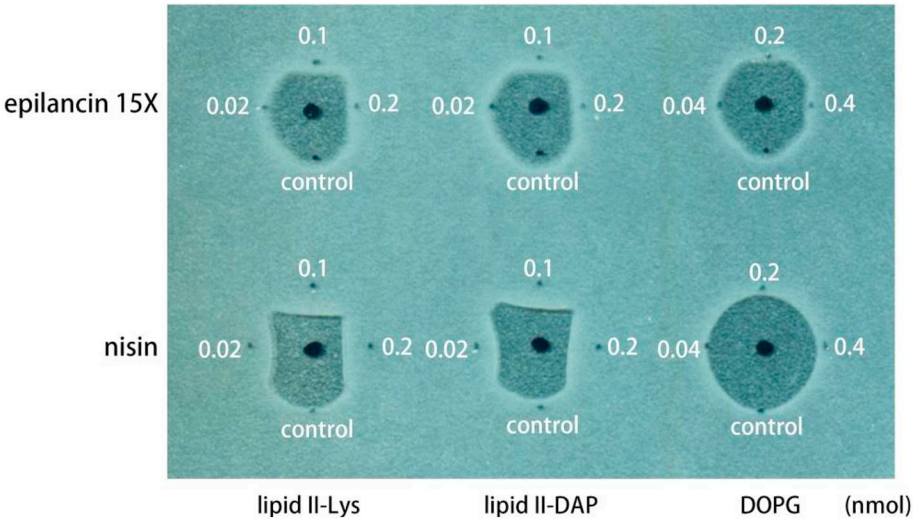

Fig. 10. Test for antagonization of epilancin 15X activity by Lipid II. Epilancin 15X and nisin are placed on the agar which is mixed with the indicator strain *Staphylococcus simulans*. Different amounts of Lipid II-Lys and Lipid-DAP (0.02–0.2 nmol) dissolved in 10 mM Tris-HCl pH 7.5, 100 mM NaCl, 0.1% Triton X-100, are spotted on the edge of the (predicted) inhibition halo and incubated overnight at 30 °C to allow growth of the indicator strain. DOPG is used as a control for the influence of electrostatic interactions only.

these peptides and in one case an arginine. At the C-terminal side of this ring there is another lysine or an arginine, but for the pep5 group this lysine is located one position further downstream. We speculate that these groups may share the same or very similar targets, which would make them quite a substantial group next to the Lipid II-targeting lantibiotics. Whether or not this is true we can only learn by identifying the target of these lantibiotics.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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