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REVIEW PAPER

A REVIEW: LANTIBIOTICS, A PROMISING ANTIMICROBIAL AGENT

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ABSTRACT

Rise in multi drug resistant bacteria has been a public health problem. This has necessitated for the exploration of novel antimicrobials. Bacteriocins, probiotic bacteria, and bacteriophages are considered as alternatives to antibiotics. To this context, lantibiotics could be the future candidate for antimicrobial agent. Lantibiotics are synthesized ribosomally and after posttranslational modification active peptide is produced. Lantibiotics are lanthionine and methyllanthionine containing peptides exhibiting activity against multi drug resistant pathogens. Besides its application as alternatives to old antibiotics, they can be used as food preservatives, additives, probiotics, and prophylactics.

Keywords: Lantibiotics, Gene cluster, Posttranslational modification, Antibiotic resistant

INTRODUCTION

Lantibiotics are antimicrobial peptides produced by most of the Gram – positive bacteria. These peptides are effective against most of the Gram-positive bacteria and few Gram-negative bacteria, for example, nisin Z at higher concentrations can affect *Escherichia coli* and other Gram-negatives like *Neisseria* or *Helicobacter pylori*. The first lantibiotic nisin was discovered in 1920s (Rogers & Whittier 1928) which is used as a safe food preservative. However, the ribosomal origin of such modified peptides was revealed only after 60 years, when epidermin biosynthetic gene cluster from *Staphylococcus epidermidis* Tü 3298 was sequenced (Schnell *et al.* 1988). Research on lantibiotics has gained renewed interest due to the emergence of antibiotic resistant strains such as, methicillin resistant *Staphylococcus aureus* (Rubin *et al.* 1999), vancomycin resistant *Enterococcus faecium*, multi drug resistant Gram-negative pathogens - *Pseudomonas aeruginosa*, *Acinetobacter baumanii* and Enterobacteriaceae (Livermore 2004) etc. Since then, hundreds of lantibiotics have been described, and most of them are produced by Gram-positive bacteria (Emma *et al.* 2013). Likewise, many more lanthipeptide gene cluster have been characterized from actinomycetes viz- cinnamycin from *Streptomyces cinnamoneus* DSM 40005 (Widdick *et al.* 2003), SapB from *Streptomyces coelicolor* (Kodani *et al.* 2004), SapT from *Streptomyces tendae* (Kodani *et al.* 2005), actagardine from *Actinoplanes garbadinensis* (Boakes *et al.* 2009), deoxyactagardine B (DAB) from *Actinoplanes liguriae* (Boakes *et al.* 2010), venezuelin from *Streptomyces venezuelae* (Goto *et al.* 2010), and microbisporicin from *Microbispora corallina* (Foulston *et al.* 2010). Furthermore, lanthipeptide gene clusters are also found in proteobacteria, chlamydiae, bacteroidetes and cyanobacteria (Li *et al.* 2010, Marsh *et al.* 2010).

Biosynthetic gene cluster

Lantibiotics are synthesized ribosomally and post translationally modified (Schnell *et al.* 1988, Arnison *et al.* 2012).The genes required for the biosynthesis of lantibiotics are arranged in cluster. The genes are designated by generic locus *lan* (eg. *nis* for nisin (Gross & Morell 1971), *gdm* for gallidermin (Kellner *et al.* 1988), *pse* for pseudomycoicidin (Basi-Chipalu *et al.* 2015) etc. Gene clusters can be found on conjugative transposable elements (e.g., nisin), on the chromosome of the host (e.g., subtilin), or on the plasmids (e.g., epidermin, lacticin 481) (Chatterjee *et al.* 2005). The lantibiotics are characterized by Lanthionine (Lan), methyllanthionine (MeLan) and other modified amino acids such as didehydroalanine (Dha) and didehydrobutyrine (Dhb) (Schnell *et al.* 1988). The representative gene clusters for biosynthesis of specific lantibiotics are shown in fig 1 (see appendix). The

gene cluster consist of structural gene *lanA* that encodes prepeptide, modification gene *lanB,C,M, L, labKC* encoding enzymes that introduce thioether rings, transporter (*lanT*) gene that exports modified peptide as well as cleaves leader peptide, and / or extracellular protease (*lanP*) that removes the leader, and immunity gene [*lanI (H)* and/or *lanFEG*] that protect the producer bacteria from its own product. Normally, parts of gene cluster are organized in the form of operon. The structural gene is often clustered as first ORF. Besides this, there is no uniform gene order in the individual gene clusters (Siezen *et al.* 1996, Willey & van der Donk 2007). Other genes that are necessary for modification, transport, processing, immunity, and regulation are arranged close to the structural genes.

The regulation of lantibiotic biosynthesis is done either by a quorum-sensing or by growth phase dependent mechanisms (Chatterjee *et al.* 2005). Quorum sensing system consists of a receptor-histidine kinase (*lanK*) and its cognate transcriptional response regulator (*lanR*) (McAuliffe *et al.* 2001, Bierbaum & Sahl 2009). The active lantibiotic peptide acts as a triggering molecule which leads to a signal cascade initiated by autophosphorylation of the *lanK* histidine residue (Kuipers *et al.* 1995, Schmitz*et al.* 2006). Subsequently, the phosphate group is transferred to the LanR that often functions as a transcriptional activator that ultimately regulates transcription of the target genes (van Kraaij *et al.* 1999, Yonezawa & Kuramitsu 2005, Willey & van der Donk 2007).

Classification of Lantibiotics

Recently, lantibiotics are classified on the basis of their biosynthetic pathway (Willey & van der Donk 2007, Knerr & van der Donk 2012). In Class I lantibiotics (eg. nisin), *lanB* dehydratase catalyses dehydration of Ser or Thr to didehydroalanine (Dha) and Z-didehydrobutyrine (Dhb), respectively. *lanC* cyclases catalyses the formation of the characteristic lanthionine (Lan from Ser) and methyllanthionine (MeLan, from Thr) thioether crosslinks by intramolecular addition of Cys thiols to Dha/Dhb. Then ABC transporter *lanT* transports the mature prepeptide and protease *lanP* cleaves the leader peptide (van der Meer *et al.* 1993, Meyer *et al.* 1995). In class II lantibiotic (eg. mersacidin), both dehydration and cyclization steps are catalysed by a single enzyme lanM (Siezen *et al.* 1996). In class III lantibiotics (Sap B and Sap T), the peptides are modified by RamC. These peptides lack antimicrobial activity; instead they perform morphogenic and signaling functions for the producer cells. Likewise, the labyrinthopeptins are also categorized under the class III lantibiotics, because they are also posttranslationally processed by the RamC-like kinase-cyclase Lab KC enzyme (Meindl *et al.* 2010). The gene clusters of the labyrinthopeptins also possess two *lanT* like transporters without a dedicated protease (Müller *et al.* 2011, Knerr & van der Donk 2012). In class IV lanthipeptides (eg. venezuelin), the peptides are modified by the lanthionine synthetase *lanL* (Goto *et al.* 2010). Similar to the enzymes of the class III lantipeptides, it also has a *lanT*-like transporter, but no dedicated protease or protease domain (Goto *et al.* 2010, Knerr & van der Donk 2012).

Post-translational modification

Initially lantibiotics are synthesized as inactive prepeptide which is later converted to active peptide by extensive post translational modifications. The post translational modifications include the dehydration of serine and threonine residues to form the didehydro amino acids Dha and Dhb (Fig. 2), respectively (see appendix). This step is catalyzed by modification enzyme (*lan B/M/ L/*Lab KC). The Dha and Dhb residues later react with the nearby C-terminally located Cys residues to form thioether rings - *lan* and MeLan, respectively which is catalyzed by *lanC/lanM* (Tang & van der Donk 2012, 2013). Final step is catalyzed by *lanT/lanP*, which exports the modified peptide and cleaves its leader peptide to produce an active form.

Mode of action

Lantibiotics kill the target cells by different mechanisms (Asaduzzaman & Sonomoto 2009). Lantibiotics show activity against Gram-positive bacteria while not effective against Gram-negative bacteria (Castiglione *et al.* 2008). Generally, Gram-negatives are not affected by lantibiotics due to the presence of protective outer membrane which restrict the entry of lantiobiotics into the cytoplasmic membrane. However some lantibiotics like nisin Z can affect Gram negatives – *Escherichia coli*, *Neisseria* or *Helicobacter pylori* at higher concentration. The activity of this lantibiotic might be due two mechanisms: i. the self promotion uptake, ii. the destabilization of the outer membrane by binding of lantibiotics to the lipopolysachharides (Nagao *et al.* 2009). Similarly,

the lantibiotic produced by *Bifidobacterium longum* DJO10A has a prospect to inhibit members of the Enterobacteriaceae. In addition, microbisporicin from *Acinetobacter* also exhibited the activity against Gram-negative bacteria such as *Moraxella catarrhalis*, *Neisseria* sp., and *Haemophilus influenza* (O'Sullivan & Lee 2011). The mode of action of lantibiotics is primarily based on two mechanisms: pore formation and inhibition of peptidoglycan synthesis (Brötz *et al.* 1998b).

Pore formation

Pore formation is one of the important mechanisms of lantibiotics for its antimicrobial activity. The pores formed by the lantibiotics may have the diameter of 2 nm and the pores' half life is about few to several hundred milliseconds (Brötz *et al.* 1998). Many lantibiotics make a lipid II mediated pores in the target bacteria (Brötz *et al.* 1998). In this context, lipid II seems to enable the peptides to integrate into the membrane (van Heusden *et al.* 2002) as well as stabilizes the resulting pores (Breukink *et al.* 1999, Wiedemann *et al.* 2004). Nisin is the one in which there has been an extensive study on the mechanism of pore formation. Ramseier in 1960 showed the first evidence on pore formation. He observed a leakage of intracellular compounds from clostridial cells treated with nisin. In addition, Brötz *et al.* (1998b) and Breukink *et al.* (2003) also showed that nisin binds to lipid II, using it as a docking molecule to form a stable and efficient pore. Later, Hsu and colleagues described that, nisin binds to the pyrophosphate moiety of the lipid II by five hydrogen bonds (Hsu *et al.* 2003). This interaction results in the insertion of the elongated C-terminus of nisin into the membrane (van Heusden *et al.* 2002; Hasper *et al.* 2004). This insertion causes an efflux of ions and small cytoplasmic compounds that leads to the dissipation of the membrane potential. Nisin is said to cause both mechanisms (pore formation and inhibition of cell wall biosynthesis) for its antimicrobial activity, however, pore formation is the primary cause. This could be explained by the rapid killing of cells by pore formation before inhibition of peptidoglycan synthesis could take place (Brötz *et al.* 1998b, Wiedemann *et al.* 2001).

Mechanism of pore formation by binding to lipid II is also shown by two peptide lantibiotics *viz* haloduracin (Oman *et al.* 2011) and lacticin 3147 (Morgan *et al.* 2005). In case of lacticin 3147, first A1 peptide binds to the lipid II and forms a complex. Later, the A2 peptide binds to the complex of peptide A1 and lipid II and forms a trivalent complex. This trivalent complex leads to the deeper insertion of the complex into the membrane (Deegan *et al.* 2006, Wiedemann *et al.* 2006 Oman & van der Donk 2009). This complex arrangement allows the A2 peptide to adopt a transbilayer orientation, thereby resulting in the pore formation. These pores are comparatively smaller (0.6 nm) than those formed by the nisin (Bonelli *et al.* 2006). Haloduracin also forms pores by similar mechanism as shown by lacticin 3147 (Oman *et al.* 2011). Streptococcin A-FF22 and Pep5 are example of lantibiotics that can form unstable pores. These pores dissipate the membrane potential but do not release the large molecules from the cell (Kordel *et al.* 1988, Jack *et al.* 1994). Likewise, small peptides such as gallidermin and epidermin can also form pores that depend on the thickness of the bacterial membrane (Bonelli *et al.* 2006). Thus, the antibacterial activity of epidermin and gallidermin may not always be due to the pore formation but could also be due to inhibition of peptidoglycan synthesis (Xiulan *et al.* 2012).

Inhibition of peptidoglycan biosynthesis

The inhibition of peptidoglycan biosynthesis is another important mode of action of lantibiotics. Lipid I and lipid II are the essential precursors for cell wall biosynthesis (Linett & Strominger 1973). Lantibiotics bind to lipid I and lipid II, the essential precursors for cell wall biosynthesis. This binding makes the transpeptidase and transglycosylase unable to utilize lipid II, which ultimately inhibits the peptidoglycan biosynthesis.

Nisin is able to accumulate the lipid II thus inhibits the synthesis of peptidoglycan (Reisinger *et al.* 1980). The ability of the nisin and epidermin to inhibit the conversion of lipid I to lipid II was described by Brötz *et al.* in 1998. Likewise, in the nisin-like lantibiotics having conserved two N-terminal lanthionine rings dissipate the lipid II from its functional location in the cell wall biosynthesis complex (Hasper *et al.* 2006). This dissipation of lipid II results in the accumulation of UDP-linked peptidoglycan precursors in the cytoplasm, thus inhibiting peptidoglycan synthesis (Castiglione *et al.* 2007, 2008).

Mersacidin, actagardine, and cinnamycin are example of other lantibiotics that also inhibits cell wall biosynthesis by binding to lipid II, specifically bind to transglycosylases (Brötz *et al.* 1997, Hsu *et al.* 2003). The lantibiotics of the

mersacidin group target the acetylglucosamine moiety and most probably the sugar and phosphate residues of the lipid II. All mersacidin-like lantibiotics possess a conserved TxS/TxEC motif (Hsu *et al.* 2006, Böttiger *et al.* 2009). Especially, the Glutamate residue in the motif is essential for the antibacterial action of mersacidin (Szekat *et al.* 2003). Moreover, the bioactivity of mersacidin is ion dependent, since, the presence of calcium ions increases its activity *in vivo*. Since lipid II is negatively charged, calcium ions can transfer a net positive charge to the neutral peptides like mersacidin, which might result in enhanced membrane interaction and consequently in deeper membrane insertion (Böttiger *et al.* 2009). Plantaricin C, a positively charged peptide in which calcium might stabilize the conformation that promotes the membrane insertion. An ion independent mode of action was observed for an uncharged lacticin 481. It contains a positively charged amino acid on its N-terminus that might be sufficient for membrane interaction and formation of lipid II complexes (Böttiger *et al.* 2009).

Other functions

Many lantibiotics have other biological functions than pore formation and hindrance on peptidoglycan biosynthesis. Lantibiotics like SapT and SapB exhibit a morphogenetic rather than an antibacterial effect. Because of their amphiphilic nature, these peptides serve as the biosurfactants, facilitating the emergence of aerial hyphae (Kodani *et al.* 2004, 2005). Nisin and Pep5 induce autolysis of certain staphylococcal strains, leading to a break down of the cell wall at the septa of dividing cells (Bierbaum & Sahl, 1987). Likewise, nisin, subtilin and sublancin inhibit the spore outgrowth from *Bacillus* and *Clostridium* species (Hurst, 1981, Paik *et al.* 1998). The lantibiotics of cinnamycin subgroup (duramycin, duramycin B, duramycin C, cinnamycin and ancovenin) shows antibactericidal activity only against a few bacterial strains and *Bacillus* sp. Specifically, duramycin impairs ATP dependent protein translocation (Chen & Tai 1987) and interferes with calcium uptake (Navarro *et al.* 1985). In addition, duramycin also blocks the transport of chloride, sodium and potassium (Xie *et al.* 1983, Stone *et al.* 1984) and inhibits the proton pump of clathrin coated vesicles (Nakamura *et al.* 1984). Likewise, nukacin ISK-1, also a lantibiotic, exhibit a bacteriostatic mode of action rather than a bactericidal. It does not affect the membrane potential or form pores, but it reduces the width of the cell wall, causing incomplete formation of the septum, and thus preventing active growth (Assaduzzaman & Sonomoto, 2009).

Application of lantibiotics

Lantibiotics have vast array of application is areas like, food industy, medicine, health care, etc. The characteristics like low molecular weight , a broad range of antimicrobial activity, lack of toxicity and low immunogenicity (Asaduzzaman & Sonomoto, 2009, Dischinger *et al.* 2014) make them applicable in multiple areas. Nevertheless, lantibiotics need to overcome some obstacles before they can be used in the medical applications. For example, Nisin has few drawbacks such as a low stability at the physiological pH of the gastrointestinal tract, a low solubility, and has the tendency to interact with the blood components (Dischinger *et al.* 2014).

At present there is only one lantibiotic, nisin, which has been used commercially. It has been used as a powerful and safe food preservative in processed dairy products, canned fruits and vegetables since it has no known toxicity to humans (Delves-Broughton 1990). Nisin in addition exhibits an antimicrobial activity against food spoilage bacteria like *Listeria monocytogenes* (Cotter *et al.* 2005). It is applied in veterinary medicine as well for the treatment of bovine mastitis (Broadbent *et al.* 1989). Furthermore, as nisin is effective against clinically relevant human pathogens, like *Helicobacter pylori*, it might be an effective drug in peptic ulcer treatment too. Moreover, it is also used in treatment of oral decay, enterococcal infections and treatment of enterocolitis (Ryan *et al.* 2002, Nascimento *et al.* 2006). Other applications of nisin include the inhibition of experimental vascular graft infections caused by methicillin-resistant *Staphylococcus epidermidis* (Ghiselli *et al.* 2004). In addition to its antibiotic effect, nisin also inhibits sperm motility, showing its potential as a contraceptive agent (Aranha *et al.* 2004).

Apart from nisin, many other lantibiotics have also been investigated for their possible applications as antimicrobials. Mersacidin and actagardine show a notable activity against methicillin resistant *Staphylococcus aureus* (MRSA) infection, bacterial mastitis, oral decay, acne, etc (Limbert *et al.* 1991). Likewise, gallidermin and epidermin are affective against acne, eczema, follicultis, and impetigo, thus, they might be used for personal care products. Cinnamycin might be used against inflammation and viral infections, and for blood pressure

regulation (Ryan *et al.* 2002). Similarly, Pep5 and epidermin prevent the adhesion of coagulase-negative staphylococci, specifically *S. epidermidis*, to siliconisated catheters (Fontana *et al.* 2006). Mutacin 1140 may prevent dental cavities. Duramycin and ancovenin can be used for inflammation and blood pressure regulation respectively. Lacticin 3147 prevents *Propionibacterium acnes* from causing acne, thus these substance may also be used as additives in cosmetics and personal care products (Kellner *et al.* 1988, Lawton *et al.* 2007). Salivaricins are effective against *Streptococcus pyogenes* strains. Therefore, the salivaricin A producer was supplemented as a probiotic to milk drinks. Moreover, chewing gums and lozenges containing salivaricin-producing strains have been developed (BLIS Technologies).

There are several actinomycete lanthipeptides described having clinical applications. NVB302 (an actagardine derivative) and Moli1901 (also known as lancovutide or duramycin, a structural analogue of cinnamycin) successfully completed Phase I and Phase II clinical trials for the treatment of *Clostridium difficle* infections (Crowther *et al.* 2013) and cystic fibrosis (Grasemann *et al.* 2007, Oliynyk *et al.* 2010). Likewise, NAI-107 (also known as microbisporin) is in a late stage of preclinical development for the treatment of multi drug resistant Gram-positive pathogens (Jabes *et al.* 2011). Furthermore, lantibiotics could be used in food products. McAuliffe *et al.* (1999) and Rodriguez *et al.* (2001) had described the possible use of starter cultures of Lacticin 3147 or lacticin 481 producing *Lactococcus lactis* to inhibit *Listeria monocytogenes* in cheese production.

CONCLUSION

Lantibiotics have a huge potential as alternatives for antimicrobial drugs. Till date, only nisin is used commercially as a food preservative. Although many lantibiotics are under clinical and preclinical trials to explore them as therapeutics, none is yet known to have approved for use as pharmaceuticals. Apart from its use as an agent against multi drug resistant bacteria, it could also be used for personal care products, veterinary medicine, and other biotechnological purpose. Currently, there is a very limited knowledge on mode of action of lantibiotics which yet need to be explored thoroughly. Bioinformatics data with subsequent protein engineering could help discover more lantibiotics.

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REFERENCES

Altena, K., Guder, A., Cramer, C. and Bierbaum, G. 2000. Biosynthesis of the lantibiotic mersacidin: organization of a type B lantibiotic gene cluster. *Applied and Environmental Microbiology* **66** (6): 2565–2571.

Aranha. C., Gupta, S. and Reddy, K.V. 2004. Contraceptive efficacy of antimicrobial peptide Nisin: *in vitro* and *in vivo* studies. *Contraception* **69** (4): 333–338.

Arnison, P. G., Bibb, M. J., Bierbaum, G., Bowers, A. A., Bugni, T. S., Bulaj, G., Camarero, J.A., Campopiano, et al. 2012. Ribosomally synthesized and post-translationally modified peptide natural products: overview and recommendations for a universal nomenclature. *Natural Product Reports* **30**(1): 108–160.

Asaduzzaman, S. M. and Sonomoto, K. 2009. Lantibiotics: diverse activities and unique modes of action. *Journal of Bioscience and Bioengineering* **107** (5): 475– 487.

Basi-Chipalu, S., Dischinger, J., Josten, M., Szekat, C., Sahl, H. G. and Bierbaum, G**.** 2015 Pseudomycoicidin, a class II lantibiotic from Bacillus. *Applied Environmental Microbiology* **81**(10):3419-3429.

Bierbaum. G. and Sahl, H. G. 1987. Autolytic system of *Staphylococcus simulans* 22: influence of cationic peptides on activity of N–acetylmuramoyl–L–alanine amidase. *Journal of Bacteriology* **169** (12): 5452–5458.

Boakes, S., Appleyard, A. N., Cortes, J. and Dawson, M. J**.** 2010. Organization of the biosynthetic genes encoding deoxyactagardine B (DAB), a new lantibiotic produced by *Actinoplanes liguriae* NCIMB41362. *The Journal of Antibiotics (Tokyo)* **63** (7): 351–358.

Boakes, S., Cortes, J., Appleyard, A. N., Rudd, B. A. and Dawson, M. J. 2009. Organization of

the genes encoding the biosynthesis of actagardine and engineering of a variant generation system. *Molecular Microbiology* **72** (5): 1126–1136.

Boettiger, T., Schneider, T., Martinez, B., Sahl, H. G. and Wiedemann, I. 2009. Influenceof Ca2+ions on the activity of lantibiotics containing a mersacidin-like lipid IIbinding motif. *Applied Environmental Microbiology* **75**, 4427–4434.

Bonelli, R. R., Schneider, T., Sahl, H. G. and Wiedemann, I. 2006. Insights into *in vivo* activities of lantibiotics from gallidermin and epidermin mode of action studies. *Antimicrobial Agents and Chemotherapy* **50** (4): 1449–1457.

Breukink, E., van Husden, H. E., Vollmerhaus, P. J., Swiezewska, E., Brunner, L., Walker, S., et al. 2003. Lipid II is an intrnsic component of the pore induced by nisin in bacterial membranes. *The Journal of Biological Chemistry* **278**(22): 19898–19903.

Breukink, E., Wiedemann, I., van Kraaij, C., Kuipers, O. P., Sahl, H. G. and de Kruijff, B. 1999. Use of the cell wall precursor lipid II by a pore-forming peptide antibiotic. *Science* **286** (5448): 2361–2364.

Broadbent, J. R., Chou, Y. C., Gillies, K. and Kondo, J. K. 1989. Nisin inhibits several Gram-positive, mastitis-causing pathogens. *Journal of Dairy Science* **72** (12): 3342–3345.

Brötz, H., Bierbaum, G., Leopold, K., Reynolds, P. E. and Sahl, H. G. 1998a. The lantibiotic mersacidin inhibits peptidoglycan synthesis by targeting lipid II. *Antimicrobial Agents and Chemotherapy* **42** (1):154–160.

Brötz, H., Bierbaum, G., Reynolds, P. E. and Sahl, H. G. 1997. The lantibiotic mersacidin inhibits peptidoglycan biosynthesis at the level of transglycosylation. *European Journal of Biochemistry* **246** (1): 193–199.

Brötz, H., Josten, M., Wiedemann, I., Schneider, U., Götz, F., Bierbaum, G. et al. 1998b. Role of lipid-bound peptidoglycan precursors in the formation of pores by nisin, epidermin and other lantibiotics. *Molecular Microbiology* **30** (2): 317–327.

Castiglione, F., Lazzarini, A., Carrona, L., Corti, E., Ciciliato, I., Gastaldo, L., 2008. Determining the structure and mode of action of microbisporin, a potent lantibiotic active against multiresistant pathogens. *Chemistry and Biology* **15** (1): 22–31.

Chatterjee, C., Paul, M., Xie, L. and van der Donk, W.A. 2005. Biosynthesis and mode of action of lantibiotics. *Chemical Reviews* **105** (2): 633–684.

Chen, L. and Tai, P. C. 1987. Effects of antibiotics and other inhibitors on ATP dependent protein translocation into membrane vesicles. *Journal of Bacteriology* **169** (6): 2373–2379.

Cotter, P. D., Hill, C. and Ross, R. P. 2005. Bacterial lantibiotics: strategies to improve therapeutic potential. *Current Protein and Peptide Science* **6** (1): 61–75.

Crowther, G. S., Baines, S. D., Todhunter, S. L., Freeman, J., Chilton, C. H. and Wilcox, M. H. 2013. Evaluation of NVB302 versus vancomycin activity in an *in vitro* human gut model of *Clostridium difficile* infection. *Journal of Antimicrobial Chemotherapy* **68** (1): 168–176.

Deegan, L., Hill, C., Ross, P. and Sahl, H. G. 2006. The mode of action of the lantibiotic lacticin 3147 - a complex mechanism involving specific interaction of two peptides and the cell wall precursor lipid II. *Molecular Microbiology* **61** (2): 285–296.

Delves-Broughton, J. 1990. Nisin and its uses as a food preservative. *Food Technology* **44** (3): 100–117.

Dischinger, J., Basi-Chipalu, S. and Bierbaum, G**.** 2014. Lantibiotics: promising candidates for future applications in health care. *International Journal of Medical Microbiology* **304** (1): 51–62.

Sherwwood, E. J., Hesketh, A. R. and Bibb, M. J. 2013. Cloning and analysis of the planosporicin lantibiotic biosynthetic gene cluster of *Planomonospora alba. Journal of Bacteriology* **195** (10): 2309–2321.

Fontana, M. B., de Bastos Mdo, C. and Brandelli, A. 2006. Bacteriocins Pep5 and epidermin inhibit *Staphylococcus epidermidis* adhesion to catheters. *Current Microbiology* **52** (5): 350–353.

Foulston, L. C. and Bibb, M. J. 2010. Microbisporicin gene cluster reveals unusual features of lantibiotic biosynthesis in actinomycetes. *Proceeding of National Academy of Science USA* **107**: 13461–13466.

Ghiselli, R., Giacometti, A. and Cirioni, O. 2004. RNAIII-inhibiting peptide and/or nisin

inhibit experimental vascular graft infection with methicillin-susceptible and methicillin resistant *Staphylococcus epidermis*. *European Journal of Vascular and Endovascular Surgery* **27** (6): 603–607.

Goto, Y., Li, B., Claesen, J., Shi, Y., Bibb, M. J. and van der Donk, W.A. 2010. Discovery of unique lanthionine synthetases reveals new mechanistic and evolutionary insights. *PLoS Biology* **8** (3): e1000339.

Grasemann, H., Stehling, F., Brunar, H., Widmann, R., Laliberte, T. W., Molina, L., et al. 2007. Inhalation of Moli1901 in patients with cystic fibrosis. *Chest* **131** (5):1461–1466.

Gross, E. and Morell, J. L. 1971. The structure of nisin. *Journal of American Chemical Society* **93** (18): 4634–4635.

Hasper, H. E., de Kruijff, B. and Breukink, E. 2004. Assembly and stability of nisin-lipid II pores. *Biochemistry* **43** (36): 11567–11575.

Hasper, H. E., Kramer, N. E., Smith, J. L., Hillman, J. D., Zachariah, C., Kuipers, O. P., et al. 2006. An alternative bactericidal mechanism of action for lantibiotic peptides that target lipid II. *Science* **313** (5793): 1636–1637.

Hsu, S. T. D., Breukink, E., Bierbaum, G., Sahl, H. G., de Kruijff, B., Kaptein, R., et al. 2003. NMR study of mersacidin and lipid II interaction in dodecylphosphocholine micelles. Conformational changes are a key to antimicrobial activity. *The Journal of Biological Chemistry* **278** (15): 13110–13117.

Hurst, A. 1981 Nisin. *Advanced Applied Microbiology* **27**: 85–123.

Jabes, D., Brunati, C., Candiani, G., Riva, S., Romano, G. and Donadio, S**.** 2011. Efficacy of the new lantibiotic NAI-107 in experimental infections induced by multidrug-resistant Gram-positive pathogens. *Antimicrobial Agents and Chemotherapy* **55** (4): 1671–1676.

Jack, R., Benz, R., Tagg, J. and Sahl, H. G. 1994. The mode of action of SA-FF22, lantibiotic isolated from *Streptococcus pyogens* strain FF22. *European Journal of Biochemistry* **219** (1–2): 699–705.

Kellner, R., Jung, G., Horner, T., Zahner, H., Schnell, N., Entian, K. D. et al. 1988. Gallidermin: a new lanthionine-containing polypeptide antibiotic. *European Journal of Biochemistry* **177** (1): 53–59.

Knerr, P. J. and van der Donk, W. A. 2012. Discovery, biosynthesis, and engineering of lantipeptides. *Annual Review of Biochemistry* **81**: 479–505.

Kodani, S., Hudson, M. E., Durrant, M. C., Buttner, M. J., Nodwell, J.R. and Willey, J. M. 2004. The SapB morphogen is a lantibiotic-like peptide derived from the product of the developmental gene *ramS* in *Streptomyces coelicolor*. *Procceding of National Academy of Sciences USA* 1**01**: 11448–11453.

Kordel, M., Benz, R. and Sahl, H. G. 1988. Mode of action of staphylococcin-like peptide Pep5-voltage dependent depolarization of bacterial and artificial membranes. *Journal of Bacteriology* **170** (1): 84–88.

Kuipers, O. P., Beerthuyzen, M. M., Siezen, R.J. and de Vos, W. M. 1993. Characterization of the nisin gene cluster *nisABTCIPR* of *Lactococcus lactis*. Requirement of expression of the *nisA* and *nisI* genes for development of immunity. *European Journal of Biochemistry* **216** (1): 281–291.

Lawton, E. M., Ross, R. P., Hill, C. and Cotter, P. D**.** 2007. Two-peptide lantibiotics: a medical perspective. *Mini-Reviews in Medical Chemistry* **7** (12): 1236–1247.

Li, B., Sher, D., Kelly, L., Shi, Y., Huang, K., Knerr, P.J., et al. 2010. Catalytic promiscuity in the biosynthesis of the cyclic peptide secondary metabolites in planktonic marine cyanobacteria. *Procceding of National Academy of Sciences USA* **107** (23): 10430–10435.

Limbert, M., Isert, D., Klesel, N., Markus, A., Seibert, G., Chatterjee, S., et al. 1991. Chemotherapeutic properties of mersacidin *in vitro* and *in vivo*, in: G. Jung, HG Sahl (Eds.), Nisin and novel lantibiotics, *ESCOM*, Leiden, The Netherlands. 448–456.

Linett, P. E. and Strominger, J. L. 1973. Additional antibiotic inhibitors of peptidoglycan synthesis. *Antimicrobial Agents and Chemotherapy* **4** (3): 231– 236.

Livermore, D. M. 2004. The need for new antibiotics. *Clinical Microbiology and Infection* **10** (4):1-9.

Marsh, A. J., O'Sullivan, O., Ross, R. P., Cotter, P. D. and Hill, C. 2010. *In silico* analysis highlights the frequency and diversity of type 1 lantibiotic gene clusters in genome sequenced bacteria. *BMC Genomics* **11**: 679

McAuliffe, O., Hill, C. and Ross, R. P. 1999. Inhibition of *Listeria monocytogenes* in cottage cheese manufactured with a lacticin 3147-producing starter culture. *Journal of Applied Microbiology* **86** (2): 251–256.

McAuliffe, O., O'Keeffe, T., Hill, C. and Ross, R. P. 2001. Regulation of immunity to the two-component lantibiotic, lacticin 3147, by the transcriptional repressor LtnR. *Molecular Microbiology* **39** (4): 982–993.

Meindl, K., Schmiederer, T., Schneider, K., Reicke, A., Butz, D., Keller, S., et al. 2010. Labyrinthopeptins: a new class of carbacyclic lantibiotics. *Angewandte Chemie International Edition* **49** (6): 1151–1154.

Meyer, C., Bierbaum, G., Heidrich, C., Reis, M., Süling, J., Iglesias-Wind, M.I., et al. 1995. Nucleotide sequence of the lantibiotic Pep5 biosynthetic gene cluster and functional analysis of PepP and PepC. *European Journal of Biochemistry* **232** (2): 478–489.

Morgan, S. M., O'Connor, P. M., Cotter, P. D., Ross, R. P. and Hill, C. 2005. Sequential actions of the two component peptides of the lantibiotic lacticin 3147 explain its antimicrobial activity at nanomolar concentrations. *Antimicrobial Agents and Chemotherapy* **49** (7): 2606–2611.

Müller, W.M., Ensle, P., Krawczyk, B. and Süssmuth, R. D. 2011. Leader peptide-directed processing of labyrinthopeptin A2 precursor peptide by the modifying enzyme LabKC. *Biochemistry* **50** (39): 8362–8373.

Nagao, J., Morinaga, Y., Islam, M.R., Asaduzzaman, S.M., Aso, Y., Nakayama, J. and Sonomoto, K. 2009. Mapping and identification of the region and secondary structure required for the maturation of the nukacin ISK-1 prepeptide. *Peptides* **30** (8): 1412–1420.

Nakamura, S. and Racker, E. 1984. Inhibitory effect of duramycin on partial reactions catalyzed by (Na+, K+)-adenosinetriphosphatase from dog kidney. *Biochemistry* **23** (2): 385–389.

Nascimento, J. S., Ceotto, H., Nascimento, S.B., Giambiagi-Demarval, M., Santos, K.R. and Bastos, M. C. 2006. Bacteriocins as alternative agents for control of multiresistant staphylococcal strains. *Letters in Applied Microbiology* **42** (3): 215–221.

Navarro, J., Chabot, J., Sherrill, K., Aneja, R., Zahler, S. A. and Racker, E**.** 1985. Interaction of duramycin with artificial and natural membranes. *Biochemistry* **24** (17): 4645–4650.

O'Sullivan, D. J. and Lee, J. H. 2011. Lantibiotics and uses there of. US Patent No: 7,960,505.

Oliynyk, I., Varelogianni, G., Roomans, G. M. and Johannesson, M. 2010. Effect of duramycin on chloride transport and intracellular calcium concentration in cystic fibrosis and non-cystic fibrosis epithelia. *Apmis* **118** (12): 982–990.

Oman, T. J., Boettcher, J. M., Wang, H., Okalibe, X. N. and van der Donk, W. A**.** 2011. Sublancin is not a lantibiotic but an S-linked glycopeptide. *Nature Chemical Biology* **7** (2): 78–80.

Oman, T. J. and van der Donk, W. A**.** 2009. Insights into the mode of action of the two -peptide lantibiotic haloduracin. *ACS Chemical Biology* **4** (10): 865–874.

Paik, S. H., Chakicherla, A. and Hansen, J. N**.** 1998. Identification and characterization of the structural and transporter genes for, and the chemical and biological properties of, sublancin 168, a novel lantibiotic produced by *Bacillus subtilis* 168. *Journal of Biological Chemistry* **273** (36): 23134–23142.

Ramseier, H. R. 1960. Die wirkung von nisin auf *Clostridium butyricum*. *Archives of Microbiology* **37** (1): 57–94.

Reisinger, P., Seidel, H., Tschesche, H. and Hammes, W. P. 1980. The effect of nisin on murein synthesis. *Archives of Microbiology* **127** (3): 187–193.

Rodriguez, E., Arques, J. L., Gaya, P., Nunez, M. and Medina, M. 2001. Control of *Listeria monocytogenes* by bacteriocins and monitoring of bacteriocin-producing lactic acid bacteria by colony hybridization in semi-hard raw milk cheese. *Journal of Dairy Research* **68** (1): 131–137.

Rogers, L. A. and Whittier, E. O. 1928. Limiting factors in the lactic fermentation. *Journal of Bacteriology* **16** (4): 211–229.

Rubin, R. J., Harrington, C. A., Poon, A., Dietrich, K., Greene, J.A. and Moiduddin, A. 1999. The economic impact of *Staphylococcus aureus* infection in New York City hospitals.

Emerging Infectious Diseases Journal **5** (1): 9–17.

Ryan, M. P., Hill, C. and Ross, R. P. 2002. Exploitation of lantibiotic peptides for food and medical uses. In: Peptide antibiotics - discovery, mode of action and applications. Edited by C. J. Dutton MA. Haxell HAI, McArthur RG, Wax Marcel Dekker, New York, pp193–242.

Schnell, N., Entian, K. D., Schneider, U., Götz, F., Zähner, H., Kellner, R. and Jung, G. 1988. Prepeptide sequence of epidermin, a ribosomally synthesized antibiotic with four sulphide-rings. *Nature* **333** (6170): 276–278.

Siezen, R. J., Kuipers, O. P. and de Vos, W.M. 1996. Comparison of lantibiotic gene clusters and encoded proteins. *Antonie Van Leeuwenhoek* **69** (2): 171–184.

Stone, D. K., Xie, X. S. and Racker, E. 1984. Inhibition of clathrin-coated vesicle acidification by duramycin. *The Journal of Biological Chemistry* **259** (5): 2701–2703.

Szekat, C., Jack, R. W., Skutlarek, D., Färber, H. and Bierbaum, G**.** 2003. Construction of an expression system for site-directed mutagenesis of the lantibiotic mersacidin. *Applied Environmental Microbiology* **69** (7): 3777–3783.

Tang, W. and van der Donk, W. A. 2012. Structural characterization of four prochlorosins:a novel class of lantipeptides produced by planktonic marine cyanobacteria. *Biochemistry* **51**: 4271–4279.

Tang, W. and van der Donk, W. A. 2013. The sequence of the enterococcal cytolysin imparts unusual lanthionine stereochemistry. *Nature Chemical Biology*, http://dx.doi.org/ 10.1038/nchembio.1162 Jan 13 [Epub ahead of print] PubMed PMID: 23314913).

van der Meer, J. R., Polman, J., Beerthuyzen, M. M., Siezen, R. J., Kuipers, O. P. and de Vos, W.M. 1993. Characterization of the *Lactococcus lactis* nisin A operon genes *nisP*, encoding a subtilisin-like serine protease involved in precursor processing, and *nisR*, encoding a regulatory protein involved in nisin biosynthesis. *Journal of Bacteriology* **175** (9): 2578–2588.

van Heusden, H. E., de Kruijff, B. and Breukink, E. 2002. Lipid II induces a transmembrane orientation of the pore-forming peptide lantibiotic nisin. *Biochemistry* **41** (40): 12171–12178.

Widdick, D. A., Dodd, H. M., Barraille, P., White, J., Stein, T. H., Chater, K. F et al. 2003. Cloning and engineering of the cinnamycin biosynthetic gene cluster from *Streptomyces cinnamoneus cinnamoneus* DSM 40005. *Procceding of National Academy of Sciences USA* **100** (7): 4316–4321.

Wiedemann, I., Benz, R. and Sahl, H.G. 2004. Lipid II-mediated pore formation by the peptide antibiotic nisin: a black lipid membrane study. *Journal of Bacteriology* **186** (10): 3259–3261.

Wiedemann, I., Breukink, E., van Kraaij, C., Kuipers, O.P., Bierbaum, G., de Kruijff, B. and Sahl, H.G. 2001. Specific binding of nisin to the peptidoglycan precursor lipid II combines pore formation and inhibition of cell wall biosynthesis for potent antibiotic activity. *The Journal of Biological Chemistry* **276** (3): 1772–1779.

Willey, J. M. and van der Donk, W. A. 2007. Lantibiotics: peptides of diverse structure and function. *Annual Review of Microbiology* **61**: 477–501.

Xie, L. and van der Donk, W. A. 2004. Post-translational modifications during lantibiotic biosynthesis. *Current Opinion in Chemical Biology* **8** (5): 498–507.

Xie, X.S., Stone, D. K. and Racker, E. 1983. Determinants of clathrin-coated vesicle acidification. *The Journal of Biological Chemistry* **258** (24): 14834–14838.

Xiulan, L., Daniel, J. and O'Sullivan, D. J. 2012. Contribution of the actinobacteria to the growing diversity of lantibiotics. *Biotechnology Letters* **34** (12): 2133–2145.

APPENDIX

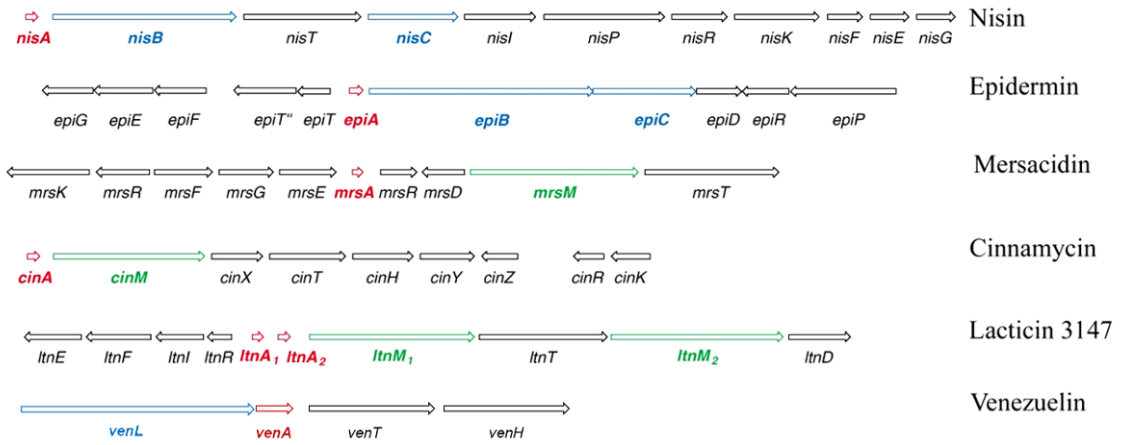

Fig. 1. Representative biosynthetic gene clusters of the lantibiotics nisin (Kuipers et al. 1993), epidermin (Schnell et al. 1992), mersacidin (Altena et al. 2000), cinnamycin (Widdick et al. 2003), lacticin 3147 (McAuliffe et al. 2001), venezuelin (Goto et al. 2010). Figure adapted from Xie and van der Donk (2004).

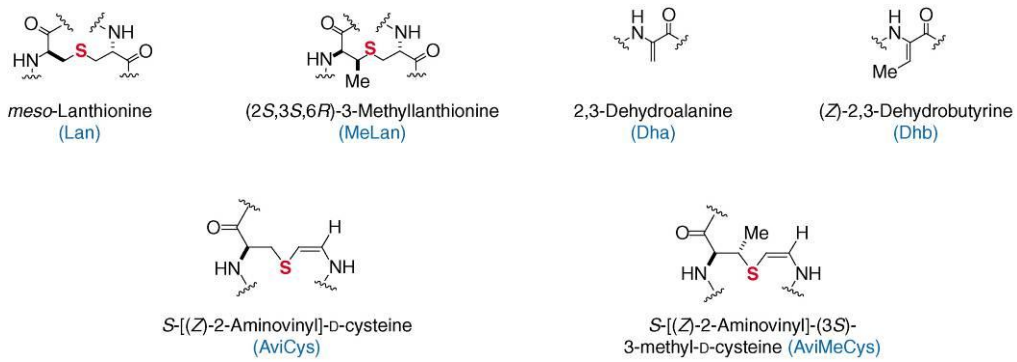

Fig. 2. Structural motifs found in lantibiotics that are introduced by post translational modification. Figure adapted from Xie and van der Donk (2004).