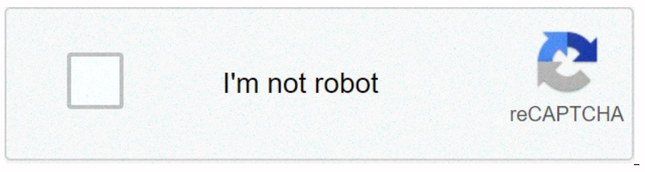

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Mode of action of bacteriocin pdf

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Bakteriocin, Lantibiotic, Nisin, Lacticin 3147, Mode of Action Bacteriocins is one of a number of antimicrobialsubstances produced by lactic acid bacteria (LAB), including organic acids, hydrogen peroxide, diacetyl and inhibitory enzymes [1, 2]. LAB has been used for centuries in the fermentation of foods, not only for taste and texture, but also because of the ability of starter-derived inhibitors to prevent the growth of spoilage and pathogenic microorganisms [3, 4]. The prototype LAB bacteriocin, nisin, was first discovered in 1928, when Rogers [5] observed metabolites of Streptococcus lactis (now reclassified as Lactococcus lactis) that were inhibitory to other LAB. The commercial application of nisin in the preservation of a number of processed foods, and the award of FDA approval in 1988 for its use as a biopreservative evoked great interest in other bacteriocins from GRAS (generally regarded as safe) organisms. These bacteriocins also have potential practical applications, and a large number of different bacteriocins have been identified and characterized in recent years. Tag et al. [6] defined bacterial hinners as 'proteinaceous compounds that kill nearby bacteria', with a bactericidal mode of action. Although many characterized bacteriocins agree with this definition, it has become apparent that some have a wide host area, which inhibits many different species. Klaenhammer [7] defined in 1993 a number of distinct classes of LAB bacteriocins; Class I bacteriocins (lantibiotics) are small (<5 kDa) peptides containing the unusual amino acids lanthionine (Lan), β-methyllanthionine (MeLan) and a number of dehydrated amino acids [8, 9]. Examples are nisin [10], lactic acid 481 [11], and the two-component antibiotics such as cytolysine produced by Enterococcus faecalis[12], lactic acid 3147 produced by L. lactis[13], and staphylococcin C55 produced by Staphylococcus aureus[14]. Class II bacteriocins are small (<5 kDa) heat-stable, non-Lan-containing, membrane-active peptides; this class is divided into Listeria-active peptides consensus sequence YGNGV (Subclass IIa), e.g. pediocin PA-1 [15], sakacin A [16], and enterocin A [17]; bacteriocins requiring two components for activity (subclass IIb), e.g. lactocostine G [18], lactic acid F [19, 20] and sec-dependent secreted bacteriocins (Subclass IIc), e.g. acidocin B [21]. Class III members are large (>30 kDa) heat-unstable proteins, e.g. helveticin J [22]. A fourth grade, the complex bacteriocins have also been proposed, requiring non-proteinaceous moieties for activity. However, this class has not been sufficiently studied at the biochemical level. In fact, experimental evidence suggests that the activities responsible for the antagonistic effects observed may be artifacts resulting from interactions between constituents from the cells and the growth medium [23]. Studies on the genetics and biochemistry of bacteriocins have mainly focused on members of Class I and II, due to the abundance of these peptides and their potential commercial applications. Class II bacteriocins have recently been extensively reviewed by others [24-27]. This review focuses on Class I bacteriocins called lantibiotics, and especially those that work by interfering with membrane integrity, i.e. type-A lantibiotics. This unique group of peptides is produced by a number of Gram-positive bacteria, and a number have been characterized at the genetic and biochemical level. 2 Molecular analysis of lantibiotics The production of ribosomally synthesized linear antimicrobial peptides is well preserved in nature, and almost all groups of organisms have been shown to produce such peptides [28, 29]. Lantibiotics (from the 'lanthionine-containing antibiotic') are unique in that they are produced on the ribosome as a prepeptide undergoing extensive post-translational modification to form the biologically active peptide [30-32]. The term is used to include peptides that contain unusual amino acids that are not normally found in nature, e.g. The presence and influence of these residues on the structure and activity of lantibiotics, and the new enzymes present in their biosynthetic pathways responsible for the specific amino acid modifications have aroused significant research interest. 2.1 Structural aspects Examples of antibiotics described so far are given in Table 1. According to a proposal from Jung in 1991 [59], lantibiotics are grouped into type-A and type-B peptides based on their structural and functional properties. In general, type-A lantibiotics are elongated, cationic peptides up to 34 residues in length that show similarities in the arrangement of their Lan bridges. These peptides work primarily by disrupting the membrane integrity of target organisms, and include nisin, subtilin, and epidermine. Type B peptides are globular, up to 19 residues in length, and work through enzyme function, e.g. inhibition of cell wall biosynthesis. Examples are the duramycins produced by Streptomyces species, mersacidin and actagardine [60]. A number of lantibiotics, however, do not fall into any category suggesting that as more are detected, classification will undoubtedly become more complex. Table 1Examples of lantibiotics characterised so far by Lantibiotic Producing strain(s) Reference Type-A Lantibioticsa Type-A(I) Nisin A L. lactis NIZOR5, 6F3, NCFB894, ATCC11454 [10] Nisin Z L. lactis N8, NIZO22186 [34, 35] Subtilin B. subtilis ATCC6633 [36] Epidermin Staphylococcus epidermidis Tu3298 [37] Gallemia Staphylococcus gallinarum Tu3928 [38] Mutacin B-Ny266 S. mutans [39] Mutacin 1140 S bribery.. s JH1000 [40] Pep5 S. epidermidis 5 [41] Epicidine 280 S. epidermidis BN280 [42] Epilancin K7 S. epidermidis K7 [43] Type-A(II) Lacticin 481 L. lactis CNRZ481, ADRIA85LO30 [11, 44] Cytolysin E. faecalis DS16 [45] Lacticin 3147 L. lactis DPC3147 [13] Staphylococcin C55 S. aureus C55 [14] Salvaricin A Streptococcus salvarius 20P3 [46] Lactocin S L. sake L45 [47] Streptococcin A-FF2 Streptococcus pyogenes FF22 [48] Sublancin 168 B. subtilis 168 [49] Carnocin U149 C. pisicola [50, 51] Variacin 8 Micrococcus variance MCV8 [52] Cypemycin Streptomyces ssp. [53] Type-B lantibiotics Cinnamycin Streptomyces cinnamoneus [54] Duramycin B Streptoverticillium ssp. [55] Duramycin C Streptomyces griseoluteus [55] Ancovenin Strepupreptitetssrept.Tomyces ssp. [56] Mersacidin B. subtilis HIL Y-85, 54728 [57] Actagardine Actinoplanes [58] 2.1.1 Unusual amino acids Lantibiotics are characterized by the presence of a high proportion of amino unusual acids, including the tenth amino acids Lan and MeLan and a number of dehydrated amino acids, such as the α,β-unsaturated amino acids Dha and Dhb [61]. This sequence-specific dehydration of serine (to Dha) and threonine (to Dhb) results in modified amino acids with electrophilic centers that can react with adjacent nucleophilic groups. The tenth lan is formed when the double bond in Dha is attacked by thiol (–SH) group of an adjacent cysteine residue [61]; MeLan results when the reaction partner is Dhb (Fig. 1). As a consequence of the presence of these intramolecular bridges, lantibiotics are polycyclic structures containing a number of Lan-rings (Fig. 2). Open in new tabDownload imageSelected structures of representative lantibiotics. Nisin A and epidermine are typically elongated, flexible peptides (type-A). Lacticin 481 represents a group with a cross-wide C-end station and an unabridged N terminal domain. Mersacidin is a type-B peptide that is conformationally well defined, globular peptides. Nomenclature of rare amino acids is based on what has previously been proposed [59]. In addition to the residues already mentioned, a number of other unusual amino acid-derived residues have been identified in antibiotics. Epidermine is a tetracyclic peptide containing unsaturated (AviCys) at the C-terminus of the mature peptide, which forms the fourth ring (Fig. 2; [62, 63]). In Pep5, Dhb occupies the N-terminus when the propeptide is split from conductor sequence [64]. However, dehydrated residues are not stable when N terminally exposed, and spontaneous deamination occurs by the addition of a water molecule, resulting in the formation of 2-oxobutyryl (from Dhb) and 2-oxypropionyl (from Dha). 2-Oxopropionyl also occurs in lactocin S, produced by Lactobacillus sake. In addition, an unusual function of lactocin S is the presence of d-alanine at three defined positions where the gene encodes a serine. It was proposed by Skaugen et al. [65] that the in vivo conversion of l-serine to d-alanine occurs by dha followed by enzymatic stereospecific hydrogenation, i.e. dha. More recently, a serine to d-alanine conversion has been reported in both peptides of the two-component lantibiotic, lacticin 3147 [13]. This was a very important finding considering that this is only the second instance of d-alanine occurring in a ribosomally synthesized peptide, and the first instance of it in a two-component biologically active peptide. It has been suggested by Ryan et al. [13] These residues may account for the broad antimicrobial inhibitory spectrum associated with milk in 3147.The presence of post-translationally modified residues in lantibiotics raises numerous questions about their function, and studies on the structure-working conditions of these peptides are becoming increasingly significant, improving our understanding of the mode of action of these unique compounds [66]. Ring conformations are believed to be crucial for maintenance of peptide stiffness [67], insensitivity to proteolytic degradation, and resistance to thermal inactivation [68]. Also, d-amino acids are known to contribute to the activity/stability of compounds [69]. However, the function of didehydroamino acids is less well defined, although it has been suggested that they may contribute to antimicrobial activity by interacting with free sulfhydryl groups on the cell envelopes of the target organism [70]. Studies on nisin showed that, while hydrolytic cleavage at Dha33 had negligible effects on biological activity, further cleavage at Dha5 resulted in significant loss of activity [71]. This is probably due to the opening of ring A in the mature nisinpeptide (Fig. 2); ring A is structurally well defined, and apparently vital for the biological activity of nisin [72]. Recently, van Kraaij et al. [73] demonstrated the importance of ring C for the biological activity of nisin Z by replacing this Lan-ring with a disulfide bond. The exchange of Ser5+ for a threonine codon in the nisin Z structural gene resulted in a nisin Z mutant housing a Dhb instead of Dha. The resulting modified peptide had a bactericidal activity 2-10-fold lower than nisin Z [67], which confirmed the importance of Dha5 in nisin. of Dha5 with an alanine did not dramatically affect the pore-forming activity of nisin against vegetative cells, but nisin Ala5 was much less effective as an inhibitor of spore outgrowth [74]. This provides strong evidence that nisin has two distinct biological activities: inhibition of bacterial growth and inhibition of spore outgrowth, produced by two distinct molecular mechanisms. The importance of ring structures in relation to biological activity has also been reported for other antibiotics [63, 75]. 2.2 Organization of lantibiotic gene clusters The events leading to the production of a lantibiotic include the formation of prelantibiotic, dehydration and cross-linking reactions, cleavage of the conductor, and excretion. In addition, the cell must be immune to the lantibiotic that it produces [60]. The genetic determinants flanking the structural gene(s) of several linear (type-A) and type-B antibiotics have so far been characterized [30, 76], and the organization of a representative number is graphically summarised in fig. 3. Comparison of gene clusters indicates the presence of a number of preserved genes proposed to encode similar functions; products of the identified genes will be discussed in detail in subsequent sections. After the generic nomenclature used for all lantibiotics, according to data from de Vos et al. [30], these include precursor peptide (LanA) and enzymes responsible for the specific modification reactions (LanB,C/LanM), accessory proteins including processing proteases responsible for the removal of leader peptide (LanP), ABC superfamily transport proteins involved in peptide translocation (LanT), regulatory proteins (LanR, K) and dedicated immunity mechanisms (LanI, FEG), in addition to genes without homologues in the database [31, 76, 77]. Open in new tabDownload slideOrganization of biosynthetic gene clusters of well-characterized lantibiotics. Structural genes (not drawn to scale) are highlighted in blue; genes with similar proposed functions are highlighted in the same colour (yellow for immunity, white for transport/processing, green for regulatory red for modification, and blue for unknown function). Gene designations are according to those Vos et et a. [30]. Two classes of genetic organization have been identified; nisine, epidermin, subtilin and Pep5 are grouped on the basis that they are modified by separate LanB and LanC enzymes, while this function is performed by a single LanM enzyme in the subclass containing lactic inspiin 481, lactic itcin S, cytolysine and mersacidin. Interestingly, the gene cluster of two-component systin 3147 contains two lanM genes. In addition, transporters with an associated protease activity have not been found in nisin-like lantibiotic gene clusters. Lantibiotic genetic determinants may be chromosomal, as in the case of subtilin [78] and SA-FF22 [79], although in most cases there are lantibiotic gene clusters on large plasmids, e.g. plasmids. [62], Pep5 [80], cytolysin [81], and lacticin 3147 [82]. Dufour et al. [83] has recently shown that the lactic acid 481 gene cluster to be present on a compound transposone, Tn5271, on a 70-kb plasmid. The Nisingens are encoded on a number of large (-70 kb) conjugative transpoons, Tn5301 from L. lactis NCFB894 [84-86] and Tn5276 from L. lactis NIZO R5 [87, 88] which also houses the sucrose utilisation genes and which are integrated into the recipient chromosome after marital transfer [7]. There is evidence that at least parts of gene clusters of some lantibiotics are organized as operon. Many consist of several transcription units [89-97] and a weak terminator structure is often found in the intergena region between the structural gene(s) and the downstream genes. Interestingly, an intragenic triple strain-loop structure has been identified within a crucial gene in the lactic acid 3147 biosynthetic operon; this structure acts to

control the level of the downstream biosynthetic genes [97]. This transcriptional organization allows moderate readthrough from the lanA promoter, ensuring a high level of transcription of prepeptide mRNA in comparison to mRNA encoding the biosynthetic enzymes. 2.3 The biosynthetic pathway 2.3.1 Prepeptides and the role of the guide sequence The (lanA structural genes) present in all lantibiotic gene clusters (nisA, epiA, etc. indicated in Fig. 3) encodes ribosomally synthesized precursor peptides called 'prepeptides'. Unlike the ripe peptides, these prelantibiotics are biologically inactive and carry an N-terminal extension, or conductor peptide, which is attached to C-terminal propeptide, e.g. nisA encodes a 57-amino-acid precursor peptide, 23 forming the conductor sequence that is split from the mature peptide in the last stage of nisin biosynthesis [98]. The propeptid domain is that which is modified and corresponds to the mature lantibiotic, but is only activated on proteolytic cleavage of the conductor. Weil et al. [99] demonstrated that in Pep5, although hydroxyamino acids serine and threonine are present in the conductor as well as propeptid, only residues in the propeptide domain undergo modification. The leader peptides of all the characterized lantibiotics are also devoid of cysteine, as opposed to cysteine-rich propeptides. The isolation of lantibiotic prepeptides from cytoplasm to produce strains has been shown to be quite difficult, suggesting that the primary translation product has a short half-life, being dehydrated immediately after synthesis [99]. Lantibiotic conductor peptides typically range from 23 to 30 amino acid residues in length and do not resemble the typical sec-dependent transport signal sequences (sec signal sequence, [100]) because they lack the hydrophobic membrane span core and the typical processing site. In accordance with de Vos et al. [30] antibiotic type-A can be classified into two groups, on the basis of size, charge, and sequence of the conductor 2.3.1.1 Type-A(I) conductors Type-A(I) conductors are generally hydrophilic, have a high proportion of charged amino acids, some of which are highly preserved, e.g. hydrophilic. This group possesses also a preserved Ser6−and Pro2−. When mutations were created in the region −18 to −15 (i.e. FNLD consensus of the nisin leader) was excretion or intracellular accumulation of nisin's immeasurable, indicating that even conservative changes in this highly preserved region lead to a block in nisin production [101]. However, similar changes in this region of pep5 leader did not eliminate activity, but production decreased significantly [102]. In addition, both groups of lantibiotics differ in the residues preceding the propeptid domain; i.e., the cleavage site. Site-directed mutagenesis at Arg1− and Ala4−, but not on the pro2−preserved, in prenisin strongly affected the cleavage of leaders and resulted in the extracellular accumulation of unprocessed, inactive nisin [101]. Consequently, these residues are most likely to be involved in leader protease recognition. The fact that unprocessed nisin was detected in the extracellular environment also indicates that cleavage of the conductor sequence is not a prerequisite for translocation. 2.3.1.2 Type-A(II) conductors Typical leader peptides type A(II) have very negative net charges; and have consensus sequences unlike those found in type A(I group, e.g. The leader sequences of type-A(II) lantibiotics, which are processed simultaneously on export, such as cytolysine [12], lactic acid 481 [11], lactic acid 3147 [82] are more similar to Class II bacteriocin conductors because they contain a double-glycine GG/GA/GS motif immediately before the cleavage site. It has been suggested that these lantibiotics and the non-Lan containing bacteriocins may be processed by a peptide ass with similar specificity [30, 103], and that the LanM type of modification enzymes occurs in this group of lantibiotics, rather than LanB/C, due to differences in the guide sequences [76]. A number of potential roles have been proposed for conductor sequences in lantibiotic prepeptides, including protection of the producer strain against high concentrations of intracellular bacteriocin since lantibiotic remains inactive with the conductor sequence attached [101]. In addition, guide peptide can play a necessary role in lantibiotic biosynthesis, in that the consensus sequences within the leader can control prepeptide towards the maturation and transport proteins. Also, it has been suggested that the conductor sequence may interact with the propeptid domain to ensure an appropriate conformation for enzyme-substrate interaction [59], given that the evidence so far suggests that changes are being made at the prepeptide stage. 2.3.2 Enzymes involved in post-translational modification Most of the genes the lantibiotic gene clusters described so far have been assigned their potential functions as a result of homology to known genes. The products of the lanB and lanC genes in the nisin-like lantibiotic systems (nisB and nisC), and the lanM genes in the systems laktikin 481 (lctM), cytolysine (cylM), lacticin 3147 (ltnM1 and ltnM2) and lactocin S (lasM) do not share sequence differences with any known proteins, and therefore their function is unclear. However, disruption of these genes in various lantibiotic systems, e.g. nisin [104, 105], epidermin [106], subtilin [107, 108], cytolysine [45], has revealed its essential role in biosynthesis, since the production of active bacteripine is abolished in their absence. It has therefore been suggested that these enzymes are strong candidates as catalysts of the new reactions responsible for the dehydration of hydroxyaminoaminoacids and ditfarna ring formation, although the molecular mechanisms involved remain to be clarified. The enzyme(s) responsible for the formation of d-alanine in lactocin S and lacticin 3147 have not yet been identified. 2.3.2.1 The LanB and LanC enzymes LanB genes encode large proteins (approximately 1000 residues), which are generally quite hydrophilic in nature, but also have some hydrophobic domains, suggesting a membrane association. This has been demonstrated for NisB, a 115-kDa protein with multiple amphibian transmembrane sphinctic α-helices, which co-sediments with membrane blisters of L. lactis[92], and also for SpaB [108]. However, it is not known whether these proteins are integrated into or simply attached to the membrane surface. In addition, EpiB was found to have a loose association with the cytoplasmic membrane [109]. An essential role has been suggested for NisB at nisinmaturation, as it has been reported that mature nisin is not excreted before NisB can be detected. The synthesis of SpaB is also strictly regulated, but conversely, occurs only when mature subtilin is detected outside the cell [110]. It has been suggested that dehydration of serine and threonine is catalyzed by LanB enzymes, although experimental evidence is lacking. In strains producing the constructed nisin variants [Trp30]nisin and [Lys27, Lys31]nisin, Ser33 is not dehydrated to Dha33 in about 50% of the total peptide produced [111]. When nisB was cloned and overexpressed in these strains, only completely modified nisin was isolated, indicating complete transformation of Ser33 to Dha33 in the mature peptide [105]. This illustrates the obvious importance of NisB in the dehydration of Ser33. Despite this fact, however, it was not possible to unequivocally assign a dehydratase function to this enzyme, since overexpression of NisB did not result in dehydration of Ser29, which remains unmodified in mature nisin. It has been suggested that the nature of the amino acids around the site of dehydration is critical for modification reactions to progress [112, 113]. The products from the LANC gene vary in size from 398 to 455 (EpiC) amino acids, and several preserved clusters can be identified within the predicted sequences. These proteins share a number of structural motifs, GXAHG, WCXG, and CHG, where the amino acids histidine and cysteine are preserved. It may be believed that these motifs have some relevance in the catalytic function of these enzymes, but no data have been produced to support this theory. Hydrophobic plots reveal alternating hydrophobic and hydrophilic regions [92], suggesting a membrane site for these proteins, as demonstrated for NisC [104], although EpiC has not been detected in membrane fractions [114]. The presumed role of these enzymes is in tioether formation, after dehydration of the prelantibiotic. Kupke and Gotz [114] reported that while purified EpiC interacted with EpiA in deceleration experiments, no modifications occurred. PepC-deficient clones were able to produce dehydrated peptides, but the majority did not contain residues and no one was right there pattern [115]. This seems to indicate an important role for pepc in titheformation, perhaps in binding on the dehydrated prepeptides and stabilizing prepeptide conformation to enable thiol groups of cysteine to react with the right dehydroamino acid. However, these observations are not incompatible with those reported for EpiC, as purified PepC was not used in this study. Overexpression of NisC did not seem to affect the dehydration of nisin prepeptides, and therefore may have a role in toitiation formation [105]. It seems that the LanB protein, along with LanC and LanT, the potential peptide translocator, is a component of a membrane-associated multimeric Lan synthetase complex, and the potentially unstable nature of this complex may explain some of the difficulties encountered in highlighting the roles of these enzymes. Using yeast's two-hybrid system, Siegers et al. [104] demonstrated the physical interaction between NisB, NisT, NisC and NisA prepeptide. While NisC co-precipitated with nisin prepeptide, nisB protein could not be detected on co-precipitation between NisB and NisA using NisA-specific antibodies. Since these antibodies were directed to the leader of NisA, it was suggested that the leader region of prepeptide is involved in NisB binding, as discussed earlier. On the basis of these and other results, a Lan synthetase complex, consisting of two molecules of NisT and NisC and a single molecule of NisB, was proposed and it is suggested that nisin prepeptide matured and moved on this membrane-bound complex. More recently, the involvement of a similar complex has been suggested in subtilin biosynthesis [116], suggesting that such arrangements could occur in all lantibiotic systems. 2.3.2.2 LanM enzymes Gene clusters of lactic acid 481, lactic cin S, mersacidin, SA-FF22 and the two-component lantibiotic cytolysine do not contain lanB or lanC type genes, but a single lanM gene a protein of 900-1000 amino acids suggested to be involved in post-translational modification of these lantibiotics. Insertion remediation of lctM (lacticin 481), cylM (cytolysine) and lasM (lactofcin S) genes respectively, led to loss of bacteriocin production phenotype [45, 95, 117], indicating an essential role of this enzyme in biosynthesis, although the specific function is still unknown. C-termini of these proteins shows striking similarity to the LanC proteins in the nisin-like lantibiotic systems, but N-termini does not show homology to the LanB class, excluding the possibility that a gene fusion between lanB and lanC gave rise to lanM. Nevertheless, it is thought that the LanM proteins combine the functions of dehydration and ditformation. Uguen et al. [118] recently used the yeast two-hybrid system to show direct contact between prepeptide of lacticin 481 and LctM; this is the first such evidence of LanM proteins and supports their function as modification enzymes. 2.3.2.3 Modification of two-component antibiotics An unusual feature of gene clusters of the two-component lantibiotic, lacticin 3147, is the presence of two ltnM genes; the requirement for two LanM proteins is unprecedented [82]. It is very likely that two lanM genes are also found in the staphylococcin C55 system, although sequencing of this gene cluster remains to be completed. It could be thought that an LtnM protein is responsible for drying the hydroxyino acids, for while other catalysed tions formation, much like the LanB and LanC enzymes in the type-A(I) group. However, the absence of a second modification enzyme in lacticin 481, lactocin S and cytolysine systems makes this unlikely. A more likely scenario is that each prepeptide requires a separate modification enzyme for activity; this has been experimentally confirmed recently for lacticin 3147 [119]. On the basis of a number of knockout experiments, it was shown that LtnM1 appears to modify the ltna1 structural time, while LtnA2 is modified by LtnM2 [119]. There is no significant sequence homology between lactic acid 3147 and cytolysine peptides, while protein sequence adjustments show that LtnA1 and LtnA2 are very closely related to the staphylocycle C55 components, SacαA and SacβA [120]. In addition, in the cytolysine system, both peptide components are much more closely related to each other than is the case for lacticin 3147 or staphylodil c55. This may explain why cytolysine can rely on only such a modification enzyme, while two LanM proteins are necessary in the lacticin 3147 system, and most likely in the staphylococcin C55 system. 2.3.2.4 Oxidative decarboxylation of EpiD EpiD, a 118-amino-acid enzyme indispensable for epidermine biosynthesis, is the only biosynthetic enzyme involved in lantibiotic formation for which a role and catalytic properties have been established. This protein is similar to a protein that has in the products of the mersacidin gene cluster [121]; It is not known if MrsD has a similar function to EpiD. Kupke et al. [122] purified EpiD and demonstrated that it is a flavo protein that requires flavin mononucleotide as a cofactor, and as such it was proposed to catalyze the oxidation reduction reaction, which is an essential step in the formation of AviCys in mature epidermine, i.e. the development of a carbon dioxide. Mass spectroscopic analysis of this new enzymatic reaction showed that EpiD catalysed oxidative decarboxylation of EpiA [123], although decarboxylation of free cysteine residues may occur spontaneously. Furthermore, this group showed that the epid recognition site is in the propeptid region of EpiA, and therefore the processing signal for this biosynthetic enzyme is not in the leader region, as has been suggested for the LanB, C and M proteins. Also, it was shown that EpiD does not react with EpiA with a C-terminal Lan present [124], and therefore, the sequence of events leading to the production of mature epidermin is most likely, dehydration and oxidative decarboxylation occurring spontaneously, followed by thieth ring formation. 2.3.3 Activation and translocation In order for a lantibiotic to exercise its antibacterial action, guide peptide, which renders prepeptidan inactive, cleaved and the mature propensitise must be translocated over the cytoplasmic membrane. Lantibiotics do not use the general secretory road [125], as they do not possess N-terminal sec-signal sequences [126]. Instead, a new system has been developed in the producers of these peptides to move the precursor over the membrane. For type-A(I) lantibiotics, proteolytic cleavage of the conductor of serine proteases [127], called LanP, is catapulted via dedicated transporters of the ABC (ATP-binding cassette)-superfamily, LanT. The type A(II) lantibiotics, which are characterized by double-glycine cleavage, have LanT transporters that split the guide peptide that accompany the export. 2.3.3.1 Proteolytic cleavage of specific leader peptideser Genes that encode products homologous to peptides have been identified in many of the lantibiotic gene clusters characterized to date. LanP proteins vary in size, from 266 amino acids (LasP) to 682 amino acids (NisP). A number of these proteins have a preprosequence, which means that the proteins are directed out of the cell, and act extracellularly. NisP is a 75-kDa protein with striking resemblance to the subtilisin-like serine proteases, and contains an N-terminal signal sequence and a C-terminal extension, LPXTGX, which could act as a membrane anchor [98]. This suggests that NisP is a secreted protein that becomes attached to the outside of the membrane, leading to speculation that cleavage of the leader peptide is the final step in nisin This was confirmed by gene discontinuation of nisP, which led to the production of completely modified nisin without antibacterial activity, since the conductor remained attached [98] The leader peptidease of the epidermin, EpiP, also possesses a signal sequence, but lacks the anchor extension observed in the NisP sequence [128], suggesting that this enzyme is exported and active outside the cell. Inactivation of epiP in Staphylococcus carnosus showed that EpiP is not an essential gene product and that this organism apparently contains a protease that can replace the epip-peptidease [128]. This also appears to be the case in subtle biosynthetic pathways, as no specific leader peptidease has been identified among the subtilin gene products. It has been suggested that a variety of secreted proteases produced by Bacillus subtilis could be involved in proteolytic activation. Contrary to the results reported for EpiP, PepP is crucial for proper cleavage of Pep5 [115]. These experiments showed that PepP is the only protease that can recognize the specific cleavage site of pre-Pep5. Although the processing of other host proteases was demonstrated, only truncated peptides were produced that showed significantly reduced biological activity. 2.3.3.2 LanT, ABC transporter The proteins responsible for moving antibiotics outside the producing cell to where they are biologically active belong to the large family of ABC transporters [129, 130]. Based on homology scans, potential transporters of this type have been found in all lantibiotic gene clusters studied so far. These translocators are characterized by four membrane-associated domains; two highly hydrophobic membrane-spanning domains, each consisting of six transmembrane regions, and two ATP-binding domains, with the preserved ATP-binding or Walker motif, GXGLST [131], on the cytoplasmic face of the membrane. Energy for export is provided by ATP hydrolysis, which probably occurs at ATP-binding domains. It is generally accepted that the membrane-spanning domains determine the substrate specificity of the carrier, which in most cases is quite relaxed [129]. Each domain can be present on a separate polypeptide; Although in almost all the antibiotics that have been characterized so far, with the exception of epidermine, both the membrane boilers and the ATP-binding domains are found on a single polypeptide. These polypeptides dimerise at the cytoplasmic membrane, forming an active migration complex. In addition, it has been shown experimentally that the transport is with the greatest faith controlled by the sequence of leaders. The function of a number of LanT proteins has been investigated through gene disorder experiments. Since it was found that inactivation of NisT led to loss of production and decreased immunity to nisin, no alternative carrier in the host appears to be able to act as a replacement for NisT [132]; however, active nisin could be detected inside producing cells. Similarly, interference with LasT [95] in loss of lactocin S production, but intracellular accumulation was not controlled. However, it seems that host-coded carriers can replace PepT in the Pep5 system [115]; although, the reported decrease in Pep5 production by approximately 10% in the absence of PepT suggests that host-provided carriers are less effective than PepT. epiT'T, found in epidermin gene clusters, would require a frameshift to provide a protein, which raises doubts about whether this protein is produced in an active form. Despite this, epidermine can be excreted in the absence of this protein [128], again probably by transporters of the host cell. Unlike the corresponding epiT, gdmT, from the natural variant of the gallidmine gene cluster, is not interrupted by an erasure [133]; expression of this gene in an epidermine producer, together with gdmH, a hydrophobic protein without known homologues, resulted in a 7-10-fold increase in epidermine production. As mentioned earlier, a second group of LanT transport proteins are present in the products of gene clusters of lantibiotics with double-glycine cleavage site. These ABC carriers have a dual function, in that they remove the conductor while translocating the substrate. Members of this family include LctT from lacticin 481 [117], CylT (also known as CylB) from cytolysine [45], LtnT from lacticin 3147 [82], ScnT from SA-FF22 [79] and MrsT from mersacidin [121]. Lactocin S is the obvious exception, as this is processed and translocated by separate LasP and LasT proteins [95]. To achieve this dual function, this new family of transporters includes a proteolytic N terminal domain belonging to the family of cysteine proteases (containing the sequence motifs QX4D/ECX2AX3MX4Y/FGX4I/L and HY/FY/VVX10I/LXDP), in addition to the integral membrane and ATP-binding domains [103]. This domain, along with the ATP-binding domain, is located inside the membrane. Similar proteins are found in many of the gene clusters of non-lantibiotic bacteriocins, such as lactococcin G [103], pediocin PA-1 [134] and plantaricin G (unpublished results cited by [103]). It is interesting to note that these bacteriocins also possess leader sequences with a double-glycine cleavage site. The spaT gene, found in the subtilin gene cluster, produces a carrier that does not have this recognized proteolytic domain, and it was widely accepted that this lantibiotic was processed by host-coded proteases. However, it was later observed that inactivation of spaT did not interfere with translocation. Interestingly, it is instead proteolytic cleavage of the conductor segment that is disturbed [135]. Sequence homologies clearly identify SpaT as an ABC carrier, and not a protease. In addition, the subtle inconductor does not have the diglycine and other motifs typical of these chimera transporters. In order for SpaT to have proteolytic activities in addition to transport activities, it would need to constitute a new class of translocators. 2.3.3.3 Activation and export of cytolysine Cytolysine is a two-component lantbibiotic, produced by E. faecalis, with both bactericidal and hemolytic biological activity. An interesting feature of the cytolysine biosynthetic pathway is the presence of both a LanP-type serine protease, called CylP (formerly CylA) [136], and a chimeric transporter with an associated proteolytic activity, called CylT (formerly CylB) [12]. CylT was the first ABC exporter to be identified in Grampositive bacteria [12]. Detailed mutagenesis studies demonstrated that while C-terminal resident ATP-binding activity CylT is not essential for CylT-mediated excretion of one of the components, CylA1, it is a prerequisite for the excretion of the other, CylA2 [45]. CylT also mediates removal of the cytolysine-subunit leader peptide during secretion [103]. In addition, CylP acts extracellularly to activate the cytolysine precursors, CylA1 and CylA2, through additional N-terminal proteolytic cleavage. Hence, a two-step process has been proposed for activation of this bacterium [137, 138]; both cytolysine precursors are externalized by CylT in a form that has an N-terminal truncation, and both cytolysine subunits are further trimmed at N-terminus by CylP in the process of activation. 2.4 Regulation of lantibiotic biosynthesis The synthesis of a number of l antibiotics has been shown to be growth-phase dependent [110, 139] and appears to be under the control of a large family of two-component signal transduction systems [140, 141]. In its simplest form, these intracellular signaling systems have two protein components: a membrane-bound sensor, a histidine protein kinase (HPK), which monitors an environmental signal; and a cytoplasmic response controller (RR) that conveys an adaptive response that is usually a change in gene expression [141]. In response to the external signal, HPK autophosphophine a preserved His residue in the C-terminal cytoplasmic domain of the protein. The phosphoryl group is then transferred to a preserved Asp of the corresponding intracellular RR, which is generally a transcription activator. Genes coding both HPKs (LanK) and RRs (LanR) have been identified in nisin gene clusters [110], subtilin [139], SA-FF22 [79] and mersacidin [121]. As regards nisin and subtilin, inactivation studies confirmed that these genes were essential for production. It is suggested that the most likely target for RR is the promoter of lanA, the lantibiotic structural gene. Although the nature of the signal molecule is unknown in the path of subtle inbiosynthesis, it has been shown that completely modified nisin autoregulates its own biosynthesis [142], by acting as a peptide pheromone for quorum sensing involving NisK and NisR [143]. A protein with some similarities to RRs, EpiQ, was shown to regulate the production of epidermin [144]; However, EpiQ does not seem to act as an RR. These, and other aspects of lantibiotic discussed below. 2.4.1 Autoregulation of nisin production As mentioned earlier, the proteins encoded by nisR and nisK have been shown to be involved in the regulation of nisin biosynthesis [98, 110]. It has recently been shown that fully modified nisin, mutant nisin species and nisin analogues can act as inducers of transcription of the nisA structural gene as well as downstream genes, via signal transduction, by acting as an extracellular signal for the sensor histidinkinase, NisK [142]. Previously, it had been reported that a 260-bp transcript was produced from the nisA gene, and that the creation of an deletion within this gene completely abolished nisA transcription [93]. ΔnisA transcription could be restored to deliver exogenous nisin in the culture medium [142]. Interestingly, unmodified prenisin, in addition to some other related lantibiotics, was unable to restore transcription, suggesting that the changes found in nisin are critical for induction. It was suggested that residues 1-11, which include the first two rings of mature nisin, may interact directly with the membrane-located NisK, thus signaling NisR to activate transcription of the nisA promoter. Gel mobility shift analyses have shown that overproduced His-tagged NisR binds to direct repetitions in the nisA promoter region, possibly as a dimer and from there, triggers gene expression [145]. 2.4.2 Regulation of lacticin 3147 immunity Analysis of lactic acid 3147 operons identified a candidate governor, LtnR; this 79-rest protein has a high degree of homology to the PBSX (Xre) family of transcriptional oppression [146]. It has recently been shown that LtnR is responsible for the repression of its own transcription and that of the downstream immunity genes, ltnIFE[97]. Regulation of expression from the promoter controlling the immunity genes (Pimm) is achieved by binding ltnR to the intergena region between ltnR and the first lacticin 3147 structural gene, ltnA1. This region includes the deviating lactic acid 3147 promoters and an inverted repeat has been identified within this region that can serve as a specific operator website. The binding of LtnR to this region results in a 90% decrease in levels of expression of the lactic acid 3147 immunity genes. It was also established that the biosynthesis of lacticin 3147 is not regulated by LtnR [97]. But while expressions from the promoter preceding the biosynthetic genes (Pbac) appear to be contitutive, an intragenic rho-independent terminator identified within the modification gene, ltnM1, probably controls the level of transcription of downstream biosynthetic genes. This transcriptional organization ensures that the right stoichiometry is maintained between the abundant prepeptide mRNA and mRNA for the biosynthetic genes. To our knowledge, this is the first report of negative regulation of gene expression by a repressive in an lantibiotic gene cluster. A regulator that appears to be has also been identified in the cytolysine gene cluster [147], but has not been further characterised. 2.4.3 Other regulatory proteins The production of epidermine is regulated by EpiQ, which possesses some similarities to RRs on the C terminus, but lacks the highly preserved phosphoryl receptor Asp residues [90]. In addition, no corresponding histidinkinase has been identified; However, it has been suggested that EpiQ may direct epidermine biosynthesis after phosphorylation of an inherent histidine kinase [106]. Despite the absence of a cognate HPK, the function of EpiQ as a transcriptional activator has been demonstrated. Peschel et al. [144] reported that EpiQ activates the epiA promoter by binding to an inverted recurrence (ANAATTACN6GTAATTNT) immediately upstream −35 region of this promoter. Similar repetitions have been identified in other promoters of epidermine gene clusters. In addition, the transcription of epiFEG, is suggested to be involved in immunity, and epiHT, putatively involved in transportation, is increased in the presence of EpiQ [133, 148]. Altena et al. [121] has recently reported identification of three regulatory genes, mrsR1, mrsR2, and mrsK2, in the mersacidin gene cluster. mrsR2 and mrsK2 are components of a two-part regulatory system that appears necessary for the transcription of mersacidin immunity soperon, mrsFGE. mrsR1 encodes a protein similar to RRs but it is unknown whether this protein plays a role in regulating the production and/or immunity of this lantibiotic. Open reading frame (ORF) 239 in the lactocin S gene cluster has a significant level of identity (22%) to WrbA, which is involved in the regulation of TRP operon expression in Escherichia coli[95]. Even in the gene cluster of Mutacin II, produced by Streptococcus mutans, a gene product with a 25% identity to a positive transcription regulator, Rgg, has been identified [149]. At present, it is not known whether these proteins play a role in regulating the biosynthesis of their respective lantibiotics. To date, regulatory proteins have not been identified in gene clusters of Pep5 or lacticin 481. 2.5 Producer immunity to antibiotics Bacteriocin production is invariably linked to the expression of specific immunity proteins required to protect the producing strain against the inhibitory action of its own product [150, 151]. To date, the mechanisms by which these proteins provide immunity are relatively unknown. Two distinct systems of lantibiotic immunity have been identified so far. The protection can be provided through the first of these, the so-called immunity proteins, LanI [89, 93, 152-156], while the second is specialised ABC transport proteins, LanFEG [79, 152, 155, 148], which can be encoded on two or three separate ORFs. 2.5.1 The immunity proteins, LanI The first LanI protein described for an lantibiotic was PepI, which is encoded by the Pep5 operon [89]. This 69-amino-acid protein has a hydrophobic N-terminal domain and a hydrophilic C-terminal part, suggesting that PepI is membrane-associated [153]. This protein is probably attached to the outer surface of the membrane where it can counteract the pore-forming activity of Pep5. In this respect, Pep5 is the immunity peptide and the proposed molecular mechanism of immunity more closely related to the immunity systems of the non-lantibiotic peptide bacteriocins [7, 150] than that of other lantibiotics. However, no suggestion of a direct interaction between Pep5 and PepI could be observed using circular dichroism (unpublished results quoted in [30]). New evidence has shown that the apparent link of immunity to the production of Pep5, which was originally reported by Reis et al. [153], is achieved by stabilizing pepI-containing transcripts through an inverted recurrence, located in wild type downstream pepA[156]. PepI shows a high degree of similarity (74.2%) to EciI, the epikidin 280 immunity protein [154], and also provides cross-immunity to epicidine 280, suggesting a similar self-protection mechanism for both antibiotics. This is the only reported case of cross-immunity between lantibiotic producers. Immunity proteins of 165 (SpaI) and 245 (NisI) amino acids are found in the gene products of the subtilin [152] and nisin [93] gene clusters. There is no sequence similarity between these proteins and PepI, except the rather hydrophobic N-terminus found in all three proteins, which can act as a membrane anchor. The presence of typical lipoprotein signal sequences [126] in both SpaI and NisI suggests that these proteins become peripheral membrane proteins, attached to the membrane of a lipid-modified N-terminal cysteine [93, 152, 157]. It is interesting to note that apart from the signal sequence and characteristic consensus cleavage site, there is no homology between SpaI and NisI, despite the subtlety and nisin sharing about 60% sequence similarity; this indicates the very specific interactions between these immunity proteins and their respective lantibiotic. Expression of nisI in both L. lactis and E. coli provided cells with a significant level of protection against exogenous nisin in liquid cultures [93] and in plate analyses [110]. However, full immunity was observed only when the mature nisin, or nisA structural gene was present [93]. This has subsequently proved to be a result of nisin autoregulation, as mature nisin is required to activate transcription of nisin's gene cluster [142]. Again, the levels of immunity that NisI assigned to these strains were only a fraction of the wild levels, providing evidence that other gene products play a role in producer self-protection in both the subtilin and nisin systems. Recently, a 116-amino-acid protein called LtnI has been shown to provide immunity to lactic acid 3147 [146]. When the ltnI gene was cloned downstream by the strong P32 promoter in pMG36e and converted to the lactic acid sensitive L. lactis MG1363, levels immunity comparable to that of the wild-type producer strain was observed. The presence of three presumed transmembrane domains suggests that LtnI is most likely localized at the cytoplasmic membrane where it may serve to prevent the insertion of the bacteriocin molecules into the membrane. Also, a leucine-zippered motif has been identified at the C-terminus of LtnI. Leucine zippers are usually associated with DNA-binding proteins where they promote dimerization [158]; It is conceivable that LtnI forms homodimers to create an active complex, but the mode of action of this protein remains to be determined. Sequence analysis of the region downstream of cytolysine activator cylP revealed a gene encoding a 327-amino-acid protein, CylI, which was shown to be essential for immunity to cytolysine [147]. A protein of similar size and hydrophobia as PepI is present in lactocin S gene cluster [95], but as yet, a role in immunity has not been demonstrated for this protein. 2.5.2. Involvement of LanFEG in immunity In addition to LanT, genes encoding a second ABC carrier have been identified in a number of lantibiotic systems; however, in this case the individual domains are encoded by separate genes. lanF encodes the intracellular ATP-binding domain, while LanE and LanG represent the membrane-boiler subunits. ABC transports of this kind are found in nisin gene clusters [155], epidermin [148], lactic acid 481 [159], and mersacidin [121]. In the subtilin system, the ABC transporter involved in immunity is coded by two genes, spaF and spaG[152]. In this case, SpaF contains both an ATP-binding domain at the N-terminus and a membrane-range domain by C-terminus [152], similar to the hisp-family of translocators [160]. In the lacticin 3147 gene cluster, genes encoding an ATP-binding domain (ltnF) and a membrane span domain (ltnE) of an ABC transporter have been identified [82, 146]; the role of these gene products in lacticin 3147 immunity remains to be investigated. Insertion inactivation of the nisFEG genes resulted in a decrease in both nisin production and immunity [155]; similar observations were reported for spaFG interruption[150]. In both cases, the mutant strains survived the relatively high amounts of bacteriocin they produced, confirming the role of other factors, most likely the LanI proteins, in providing immunity to these lantibiotics. The EpiFEG genes increased tolerance to epidermin on S. carnosus, and all three genes were required for the expression of the phenotype of immunity [148]. However, EpiFEG appeared insufficient to provide complete self-protection, suggesting that there are other immunity factors. As yet, no other presumed immunity proteins have been identified in the epidermine system. Peschel and Gotz [148] suggested that the LanFEG proteins could mediate immunity by either active extrusion of the respective peptide, which would keep the lantibiotic concentration in the membrane below critical level, or through uptake and intracellular degradation. Recently, it has been shown using peptide release assays based on HPLC analysis, that the former appears to be the case. Otto et al. [161] reported that on the incubation of cells with gagemy, the extracellular gallidermin concentration was 4-fold higher for an epiFEG-expressed strain than for a control strain, strongly suggesting that the EpiFEG transporter is functioning by expulsion of its substrate in the surrounding medium. These experiments provide the first evidence supporting the export of the respective peptide from the membrane as the molecular mechanism of immunity in strains that possess LanFEG transporters. 3 Mechanism of action for bacteriocins In general, the action of bacteriocins produced by Gram-positive bacteria is directed primarily at other gram-positive species. The range of organisms inhibited by each bacteriocin varies greatly; while nisin is active against a wide range of bacteria including strains of Lactococcus, Streptococcus, Staphylococcus, Listeria and Mycobacterium, as well as the vegetative cells and the growing spores of Bacillus and Clostridium species [2, 68, 162], the Class II bacteriocin, lactococcin A, specifically kills lactococci [162, 163]. Under normal circumstances, bacterial bacteria produced by Gram-positive bacteria do not have a bactericidal effect on gram-negative species. However, in some cases, activity against Gramnegativ may be observed on disorders of the outer membrane, as reported for nisin [165]. It has been established that the primary target for many of these small, cationic peptides is the cytoplasmic membrane of sensitive cells [164, 166-170], where they act to dissipate proton motivator force (PMF) through the formation of discrete pores in the cytoplasmic membrane, thus depriving cells of a substantial energy source [171]. PMF, which is composed of a chemical component (pH gradient; ΔpH) and an electrical component (membrane potential; Δion), drives ATP synthesis and accumulation of ions and other metabolites through PMF-driven transport systems in the membrane [172]. Collapse of PMF, induced by bacteriocin action, leads to cell death by cessation of energy-requiring reactions. Such an action has been demonstrated for type-A antibiotics and class II bacteriotins. Type-A antibiotics act in a voltage-dependent manner without requiring a specific protein receptor [173-177]; However, recent work has shown that the activity of nisin is dependent on the concentration of lipid II (undecaprenyl-pyrophosphoryl-MurNAc-(pentapeptide)-GlcNAc) in the membrane of sensitive cells [178, 179]. The Class II bacteriocins are believed to interact with membrane receptor proteins prior to insertion into cytoplasmic membranes in a voltage-independent manner [164, 167]. However, type-B antibiotics do not form membrane pores; instead, these peptides act by disrupting essential activities [180]. 3.1 Pore-forming lantibiotics Studies with intact bacterial cells, membrane bladders and artificial liposomes have shown that type-A-I antibiotics including nisin, Pep5, subtilin, lactate atin 3147 and streptococcin FF22 act to disrupt PMF, inhibit the transport of amino acids and cause the release of intracellular low molecular mass compounds, such as amino acids, ions and ATP, by forming short-lived, non-selective, transmembrane pores [177, 181–184]. It seems that in vivo, no binding to a putative target cell protein receptor is necessary for the action of the above mentioned lantibiotics, since liposomes can be used to study pore formation. The formation of such pores is energy dependent, and much of the current knowledge of the mechanism of pore formation has come from working with nisin in artificial membrane systems. 3.1.1 Biological activity of nisin As mentioned earlier, the lantibiotic nisine is bactericidal against a wide range of gram-positive bacteria, in addition to preventing the growth of spores [10]. It was shown that in vitro, nisin inhibited bacterial cell wall biosynthesis [185]. Since then, it has been shown that nisin kills bacterial cells by interfering with basic energy transduction that occurs at the cytoplasmic membrane [166, 173, 181]. It was found that pores formed in the membrane of the nisin molecules allowed diffusion of small compounds, as no transport system for ATP has been reported [173, 186]. The increase in membrane permeability results in the collapse of PMF; in the case of nisin, both ΔpHen and Δjan are completely dispersed leading to a rapid cessation of all biosynthetic processes [173, 175, 187, 188]. However, inhibition of cell wall biosynthesis is a comparatively slow process. Thus, pore formation is considered to be the primary mode of action nisin. 3.1.1.1 The effect of membrane composition The interaction between nisin with membrane components in sensitive cells is considered a vital step in its mode of action. It has been reported that even in the absence of an energized membrane, nisin may associate tightly with lipid bilayers through electrostatic interactions with the phospholipid head groups [189]. Model membrane systems have been widely used to study lipid dependence on the nisin membrane interaction [176, 189–192]. The degree of association of nisin with the membrane is largely dependent on the type of lipids present, and most importantly, the charge borne by these lipids. Several groups have shown that due to nisin's cationic nature, its activity in vitro is most effective when a high proportion of anionic, or negatively charged, membrane lipids occur [176, 193, 194]. Recent studies have substantiated this evidence, confirming the ability of nisin to insert into lipid monolayers in an anjonic lipid-dependent manner [195]. Therefore, the composition of membranes is likely an important determinant of sensitivity different bacterial species to nisin. Breukink et al. [194] reported that this initial interaction with anionic phospholipids is mediated by the C-terminal domain of the peptide, as this region contains the bulk of the positive charge carried by the nisin molecule. Replacing the valine at position 32 of nisin Z with a negatively charged glutamate residue abolished the negatively charged dependence of this interaction, reducing the antimicrobial activity of this mutant nisin Z species compared to wild-type nisin Z [194]. Thus, C-terminal domain nisin constitutes an important anjonic phospholipid binding site. Nisin also shows anionberry wave activity in carbox wetting-filled liposomes consisting of cation phospholipids [189]. Essentially, the positively charged nisin molecules bind the negatively charged carbox fluorescein molecules on the inside of the membrane, and cross the membrane like a nisin-anion complex. On the outer membrane surface, the anion is released and nisin returns to bind another molecule. This activity is strongly inhibited in liposomes consisting of anionic phospholipids, probably because the positively charged nisine is involved in interactions with the negatively charged phospholipids [176, 189]. Little or no anion carrier activity would be expected in vivo, since bacterial cell membranes are rich in anionic phospholipids. 3.1.1.2 Energy requirements for pore formation The membrane interactions described above are followed by insertion of nisin into the membrane in an energy-dependent manner. The energy state of a sensitive cell is critical for pore-forming activity of nisin; energy is required for both formation and opening of pores. The electrical transmembrane potential (Δion), generated by metabolizing bacterial cells, is considered the main driver of activity. Sahl et al. [173] reported that a Δacid is required for nisin action and similarly, studies with carbox enfluorescein-charged liposomes confirmed that a Δn is necessary for nisins membrane-interfering abilities [176]. Black-lipid membrane experiments showed that nisin can form pores only when a transnegative (inside negative) electrical potential is applied, the same orientation that occurs at the bacterial cytoplasmic membrane [173, 190]. From these experiments it was also possible to determine the threshold potential required for pore formation, and to estimate the size of the pores formed. Nisin appears to form fairly stable pores with a diameter of approximately 1 nm, with a threshold potential of approximately −80 mV [173, 190]. At low Δhinn within the range −10 to −40 mV, nisin fails to induce any permeability in artificial membranes. Nevertheless, a study has shown Δio diversion by nisin in E. coli liposomes in the absence of a threshold potential [192]. Driessen et al. [189] suggested that the presence of a Δhine may change the orientation of nisin in the membrane prior to pore formation. More recent evidence suggests that nisin is not exclusively a voltage-dependent bacteriocia. Moll et al. [196] showed the ability of nisin to divert ΔpH of L. lactis cells, even in the absence of a Δ blood cell. Therefore, while it remains so that efflux and depolarization through nisin are more effective with live cells, both Δ blood cells and ΔpH can serve to promote this activity. 3.1.1.3 Secondary mode of action Study of nisin-treated cells over a longer time course showed that this cationic peptide may induce autolysis of certain Staphylococcus species [197]. Nisin proved competitively releasing lytic enzymes from their inhibitors, polyanion cell wall constituents such as teichoic, lipoteichoic, and teichuronic acids. Under normal conditions, these enzymes, which include N-acetylmuramoyl-l-alanine-amidase and β-N-acetylglucosaminidas, strictly regulate cell wall division and circulation [198]. The non-specific activation by nisin results in the degradation of areas in the cell wall, especially in the septum range [199-201]. The combination of increased osmotic pressure, which is the result of pore formation, and a weakened cell wall encourages subsequent cell lysis. Similar observations have been reported for Pep5 [197, 199-201]. 3.1.1.4 Models for pore formation A number of tentative models have been proposed for the mechanism for pore formation of lantibiotics, but very little is yet known about the in vivo situation [202, 203]. Pore formation through the 'barrel-rod' mechanism, used by a number of cytolytic pore-forming toxins [204], has been predicted (Fig. 4). This model involves the initial accumulation of the peptide at the membrane surface through ionic interactions with the phospholipid head groups. The presence of these peptides induces significant thinning of the membrane in these areas, due to localized displacement of phospholipids. On application of a Δkon, the molecules adopt a transmembrane orientation. As lantibiotics are small peptides that can span the membrane only once, it is assumed that several molecules associate with the membrane to form a pore. Whether this aggregation of molecules occurs before insertion, or in the membrane after insertion is unknown. It has been shown that at high pH, nisin monomer aggregates outside the membrane, significantly reducing biological activity [176]. Thus, insertion is followed by aggregation the favored model. So far, it is unknown how many monomers are required to form a pore, but it is believed that it is a dynamic process with peptides that connects and leaves the pore complex. The peptides are believed to adapt around a central channel, with hydrophobic faces against lipid bilayer and hydrophilic faces towards the pore center. A wedge model has also been proposed for pore formation of nisin. Here, the positively charged C-terminus, along with the bound lipids, enters the membrane that forms a wedge-like pore consisting of several nisin molecules [189, 196, 202]. et al. [196] suggested that the hinge region of the nisin molecule may act as a twist that allows nisin to bend the lipid surface. More recently, this group has reported that nisin induces rapid movement of a fluorescent phospholipid from the inner leaflet to the outer leaflet of unilamellar phospholipid blisters [205]. This supports the theory of nisin-induced disorder of the phospholipid organization in the membrane, as suggested by the wedge-like model. There is some conflicting evidence that makes the proposal for a pore formation model difficult. Breukink et al. [206] studied the topology of nisin in model membranes using site-directed tryptophanfluorescence spectroscopy. This work showed an overall parallel orientation for nisin, suggesting that nisin causes membrane leakage by destabilizing the bilayer while being parallel to the membrane, as has been suggested for other membrane active peptides [207]. More recently, however, van Kraaij et al. [208] demonstrated that niin's C-terminus translocates the membrane, a process that would agree with the barrel-stave model described above. It was suggested that the most likely event after transient pore formation by nisin would be the movement of the entire molecule to the inside of the membrane, which has been described for magainin [209]. 3.1.1.5 Lipid II's role in nisin activity While the model described above was based on results obtained with model membrane systems, a number of observations made using intact bacterial cells remained unexplained, e.g. inthe form of a series of micromembrane systems. As mentioned earlier, it is believed that nisin also mediates inhibition of cell wall biosynthesis, by forming a complex with bactoprenol-bound peptidoglycan precursor, Lipid II [185]. Brotz et al. [211] reported that ramoplanin-treated cells of Micrococcus luteus, i.e. cells where the availability of Lipid II is reduced, were resistant to the inhibitory effect of nisin, suggesting that nisin may use Lipid II as a 'docking molecule' for binding to specific membranes. In a recent comparative study with defense peptide magainin 2 showed Breukink et al. [178] that an increase in the concentration of Lipid II in isolated model membranes, in the range of 0.001-0.1 mol percent, increases membrane sensitivity to nisin. The results of this study also showed that not only lipid II function in the activity of nisin, but that it seems to be the only goal of nisin. The effect of Lipid II was specific to nisin, suggesting a specific, high-affinity interaction of Lipid II with one or more of the structural elements of nisin, resulting in pore formation. A study that investigated the interaction between Lipid II and a number of mutated nisin species to identify structural elements in the nisin molecule involved found that mutations affecting the conformation of rings A C ([S3T]nisin) led to decreased binding of Lipid II and increased the concentration needed for pore formation [212]. However, peptides mutated in the flexible hinge region ([[N20/M21]nisin) could not form pores, but surprisingly only had a slightly reduced activity in vivo. This in vivo activity was a result of the immutable ability of this mutated nisinart to bind Lipid II, which inhibited its incorporation into the peptidoglycan chain [212]. Thus, nisin shows a Lipid-II-mediated duality, combining two killing mechanisms in one molecule and therefore the model of pore formation must be revised. Certainly, many questions related to lantibiotic-induced pore formation remain unanswered, and far more study is needed to understand these mechanisms at the molecular level. 3.1.2 Other pore-forming lantibiotics Other antibiotics that have been characterized as tension-dependent pore-for-the-former have mechanisms of action similar to those described above for nisin. Despite this, some significant differences have been reported. As previously discussed, the orientation of Δ is applied over the membrane critical for pore formation. Pep5, which nisin, forms pores only with the application of a trans-negative Δi[182]; on the other hand, subtilin, epidermin and streptococcin FF22 (SA-FF22) can act independently of the direction of potential [174, 183, 184]. Furthermore, subtilin and epidermin have been reported to form larger pores than nisin, e.g. subtilin can form pores of up to 2 nm with lifetimes of up to 10 s. On the other hand, SA-FF22 forms pores of approximately 0.5-0.6 nm in diameter and with lifetimes of only milliseconds [184]. Consequently, SA-FF22-treated cells show efflux of ions only, while it is likely that amino acids and ATP remain inside the cells. It is therefore suggested that cell death due to sa-ff22 measures is due to disturbances in PMF, rather than immediate loss of metabolites [184]. Carnocin U149, a lantibiotic produced by Carnobacterium pisicola, also acts on the cytoplasmic membrane in a similar way to nisin [50, 51]. It was observed that nisin-producing lactococci were very sensitive to inhibitory action of carnocin U149. Subsequent examination of the sensitivity of L. lactis MG1614 transformants harbouring plasmids with varying amounts of nisin biosynthetic gene cluster revealed that NisP, the proteolytic activator of nisin is the only protein in nisin biosynthesis pathway capable of improving carnocin U149 activity. Hence, NisP can act as a membrane receptor for carnocin U149 [51]. This proposed receptor-mediated action, however, appears to be specific to bacteriocin carnocin U149 and nisin-producing L. lactis strains, like other LAB, but inhibited by carnocin U149, is up to 10 times more resistant to its action than nisin-producing lactococci [51]. In addition to their ability to kill vegetative cells through pore formation, nisin and subtilin also inhibit the of bacterial endospores; however, the exact mode of inhibition remains to be clarified. It has been reported that in the case of subtilin, the molecular mechanism responsible for preventing spore outgrowth is not the same as used to inhibit vegetative cells. This activity can be clearly attributed to Dha residues at position 5. It is assumed that the double bond provides a reactive group for an interaction with a spore-related factor that is essential for outgrowth [213]. 3.2 Type-B lantibiotics 3.2.1 Subtype cinnamycin This subtype of type-B antibiotics includes cinnamycin, ankovenin and duramycins exhibiting antimicrobial activity against relatively few bacterial strains, in particular B. subtilis [214-216]. Treatment of sensitive cells with members of this bacteriocin group results in increased membrane permeability, reduced ATP-dependent calcium uptake, and ATP-dependent protein transport [217-219]. However, the mode of action of this group is not strictly limited to bacterial cells as red blood cell listening has also been observed when treated with cinnamycin. This effect could be significantly reduced by first incubating lantibiotic in the presence of the phospholipid phosphatidylkylanolamine, suggesting that cinnamycin has an ability to interact specifically with this phospholipid [220]. Similar observations were made with duramycin [221, 222]. In addition to these membrane effects, this group of lantibiotics has also been shown to inhibit the enzyme phospholipase A2, an enzyme involved in the synthesis of prostaglandins and leukotrienes in the human immune system [223]. Since phospahtidylethanolamine is the primary substrate for this enzyme, binding the lantibioticmakes the phospholipid that is not available for conversion by phospholipa A2. 3.2.2 Mode of action of mersacidin Mersacidin, a type-B-lantibiotic produced by a species of Bacillus, is active against several Gram-positive bacteria including methicillin-resistant S. aureus[224]. The mode of action of mersacidin differs not only from type-A antibiotics but also from those in the type-B group mentioned above. Treatment with mersacidin results in cessation of growth and slow listening [225]. Macromolecular biosynthetic processes are uninhibited, except for the synthesis of peptidoglycan, indicated by a block of glucose and d-alanine incorporation. In addition, the thickness of the cell wall of treated cells was markedly reduced. Thus, inhibition of cell wall biosynthesis is the primary mode of action of mersacidin [225]. Comparison of the cytoplasmic pool of peptidoglycan precursors in mersacidin-treated cells showed that the level of UDP-MurNAc-pentapeptide, the ultimate soluble peptidoglycan precursor, was unaffected by the action of lantibiotic [180], suggesting that mersacidin inhibits a later membrane-associated stage of the biosynthesis pathway. In the case of Bacillus megaterium, this step was identified as the stage, or first polymerization reaction, due to an accumulation of lipid II in the membrane (Fig. 5; [180]). Subsequent experiments have shown that the molecular basis of this inhibition is the interaction of mersacidin with lipid II, and not with the transglycosylase enzyme [226]. The existing glycapox antibiotics, such as vancomycin, inhibit transglycosylation by binding to lipid II at the peptide-side chain of this molecule [227, 228]. It is known that mersacidin does not bind to this side chain [226], or to any site directed against any antibiotic currently in use. In fact, the exact binding site of this bacteriocia remains to be identified. Thus, mersacidin and lantibiotics with similar modes of action, such as actagardine, may prove useful in the treatment of new multiresistant pathogens, such as methicillin-resistant S. aureus and vancomycin-resistant enterococci. Open in new tabDownload slideSchematic representation of the major events occurring during cell wall biosynthesis in bacteria. The probable site of inhibition of cell wall biosynthesis of mersacidin is indicated. Adapted from [203]. 3.3 Two-component bacteria A number of Class II bacteriocins requiring the complementary action of two peptides for activity have so far been characterized. These bacteriocins are referred to as the Class IIb Group, and the mode of action of some of these bacteriocins has been described. Two-component bacteria can be further classified into two types; type-E-class (Enhancing), where one of the peptides only works to enhance the activity of the other; and type-S (Synergy) class, when the activity is believed to require both peptides to complement each other [229]. 3.3.1 Lactococcin G Lactococcin G, produced by L. lactis LMG2081, was the first two-component bacterteriocin to be identified and characterized [18]. The inhibition spectrum of this bacterium includes a number of LAB and Clostridium sp. Moll et al. [170] determined that equivalent amounts of both α and β peptides were required to emit biologically active lactococcin G. This classifies lactococcin G as a type-S two-component bacteriocin [229], i.e. when present separately, neither peptide is active. It was observed that lactocostia G inhibited the uptake of amino acids, dispersed the ΔII component of PMF and reduced the intracellular level of ATP. In addition, the pH gradient was unaffected by lactococcin G-effect, which means that membranes become permeable to ions other than protons. Moll et al. [170] suggested that this was due to the formation of very specific K^+ pores in sensitive cell membranes of α and β peptides, as immediate efflux of pre-accumulated rubidium was observed on the addition of lactopsy G. Ultimate cell death is the result of a pointless cycle of ATP-driven K^+ uptake and thus, increased ATP hydrolysis [170]. More recently, it has been reported that lactococcin G also permeablises cells to Na^+ ions [230] resulting in an osmotic in treated cells and prevents Na^+-coupled transports. It is likely that lactococcin G requires a cell wall component for activity, as attempts to detect an effect on membrane vesicles and liposomes have failed [170, 230]. However, it cannot be excluded that lactococcin G acts on a transport system that works in intact cells but not in cell membrane bladders and is absent from liposomes. 3.3.2 Lacticin F Lacticin F activity occurs through the action of two peptides LafA and LafX [7, 231]. This two-component bacteriocin, produced by Lactobacillus johnsonii VPI11088, has a narrow inhibitory spectrum, exhibiting bactericidal activity against a number of Lactobacillus species and E. faecalis. Such a narrow inhibition spectrum has led to speculation that lacticin's F-action is receptor-mediated [168]. In contrast to lactococcin G, one of the peptides, LafA, has some biological activity against Lactobacillus helveticus. Thus lacticin F can be described as a type-E two-component bacteriocin [229]. Lacticin F induces membrane permeability, leading to K^+ and phosphate efflux and PMF diversion in E. faecalis cells [168], indicating that the cytoplasmic membrane is the primary target of the bactericia. Although membrane pores do not allow an efflux of ATP, intracellular ATP levels are slowly reduced in a pointless effort to regenerate PMF. Similar results were also reported using Lactobacillus delbrueckii as the target organism [168]. As with other

Class II bacteriocins, lacticin F-action appears to be PMF-independent [175]. Furthermore, the bactericidal is pH-dependent and optimal under acidic conditions. 3.3.3 Acidocin J1132 This narrow-spectrum, two-component bacteriocin, produced by Lactobacillus acidophilus JCM 1132, also works by forming pores in cytoplasmic membrane [232]. Total diversion of PMF was observed from cells of L. acidophilus JCM 2010 treated with this bacteriocin. In addition, acidocin J1132 induced efflux of glutamate, suggesting that pores large enough to allow amino acid release to form. Like lacticin F, acidocin J1132 acts in a voltage-independent manner, as efflux of glutamate could also be observed in cells that had been pre-treated with valinomycin and nigericin [232]. 3.3.4 Thermophilin 13 Unlike other two-component bacteria, thermophilin 13, produced by Termofilus for Streptococcus, has a wide host interval activity [229]. In addition, this bacteriocin was reported to form pores in cytochrome c oxidase-containing liposomes, an activity only previously observed for lantibiotics. This indicates that termofin 13 does not require a receptor, either proteinaceous or lipid, for activity. But unlike the lantibiotics, thermophilin 13 works in a voltage-independent way. Marciset et al. [229] classified thermophilin 13 as a type-E bacteriocin, as one of the peptides in isolation, ThmA, possesses bactericidal activity against S. thermophilus, C. botulinum, monocytogenes, and B. cereus. However, the activity of ThmA is amplified 40-fold when corresponding amounts of ThmB occur. 3.3.5 Lacticin 3147 The mode of action of the two-component lantibiotic lactic acid 3147 has also been established. McAuliffe et al. [177] showed that this lantibiotic exhibits bactericidal activity against a wide range of Gram-positive species, which is amplified when target cells are energized. The pores formed by lacticin 3147 were found to be selective for ions and not larger compounds like ATP. The resulting loss of ions results in immediate dissipation of Δ blood cells and hydrolysis of internal ATP, leading to δph finally collapsing and, finally, to cell death. 4 Prospects are driven mainly by the success of nisin, the huge research efforts in recent years have led to a wealth of information on both modified and unmodified bacteriocins. But despite this, the understanding of the molecular mechanisms of these peptides is far from complete. This is especially true for those antibiotics, where many questions about the function of dehydrated amino acids, the enzymatic reactions responsible for their formation, and the mechanism of action remain unanswered. The understanding of such molecular mechanisms will be particularly important in the field of biotechnology. The potential of genetic engineering for rational drug design has already been demonstrated to subtilin [75], resulting in increased stability and activity. With the further development of expression systems, the production of mutated peptides will accelerate our knowledge of the biological activities of lantibiotics. In addition, it may one day be possible to use the unique modification systems found in lantibiotic producers in vitro to design new, modified peptides that exhibit unique properties. Although the commercial exploitation of bacteriocins is so far mainly limited to nisin food applications, potential new applications for antibiotics and unmodified bacteriocins continue to develop [233]. In particular, problems such as low production levels, and instability of bacteriocins in certain foods/environments need to be addressed. In addition, these peptides must be cytolytic abilities assessed, especially in the wake of the identification and characterization of hemolysin/bacteriocin, cytolysine, produced by E. faecalis. Possibly one of the biggest obstacles to the use of other bacteriocins in food is a regulatory one. To this end, the use of bacterial cultures in food can be of great advantage over the use of purified bacterily preparations, which would be considered food additives. In these respects, bacteriocins, both modified and unmodified, deserve further, in-depth scientific attention. References [2]Ray, B. and Daeschel, M. (1992) Food biopreservatives of microbial origin. CRC Press, Boca Raton, FL.[11] () Structure, organization and expression of the lct gene for lacticin 481, a novel produced by Lactococcus lactis. , –. [13] () Extensive-translational modification, including a serine to d-alanine conversion, in two-component lantibiotic, lacticin 3147. , –. 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