Two-Peptide Lantibiotics: A Medical Perspective

Elaine M. Lawton[1], R. Paul Ross[2,3], Colin Hill[1,2,*] and Paul D. Cotter[1]

[1]*Department of Microbiology, University College Cork, Cork, Ireland;* [2]*Alimentary Pharmabiotic Centre, University College Cork, Cork, Ireland and* [3]*Moorepark Biotechnology Centre, Teagasc, Moorepark, Fermoy, Ireland*

Abstract: Lantibiotics are ribosomally synthesised, post-translationally modified antimicrobial peptides that exhibit activity against a wide-range of Gram positive bacteria. During the last decade a number of two-peptide lantibiotics, i.e. lantibiotics that function optimally as a consequence of the synergistic activity of two peptides, have been identified, six of which (lacticin 3147, staphylococcin C55, plantaricin W, Smb, BHT-A and haloduracin) are closely related. It has been established in at least one instance, i.e. lacticin 3147, that these are extremely potent antimicrobials, which are active at nanomolar concentrations against a number of microorganisms, exhibit activity against multidrug resistant nosocomial pathogens such as MRSA and VRE and significantly, to date the development of significant levels of resistance has not been apparent. Given the similarity between lacticin 3147 and related two-peptide lantibiotics, it is likely that they too possess similarly beneficial traits and thus could potentially have medical and veterinary applications. In addition to discussing these aspects of two-peptide lantibiotic reasearch, this review will focus on new developments in this area pertaining to studies elucidating the mechanism of action of these antimicrobials, the use of bioengineering to reveal the location of essential and variable domains therein and the potential for the use of *in vivo* and *in vitro* engineering to create derivatives with even greater activities against specific target organisms.

INTRODUCTION

The study of two-peptide lantibiotics represents a relatively new field of science. However these antimicrobials, active through the synergistic activity of two peptides, represent excellent models for the investigation of peptide-peptide interactions, peptide-receptor interactions, post-translational modification of peptides and membrane-pore formation. Significantly, with respect to this review, it has already been established that as a consequence of their broad range of activity two-peptide lantibiotics exhibit great potential as antimicrobial agents.

All lantibiotics, including those requiring two-peptides, belong to the class I bacteriocins. For centuries we have inadvertently benefited from the production of bacteriocins by generally regarded as safe bacteria in foods and their associated bio-preservative effects [1]. From an even earlier period of history the production of bacteriocins by commensal bacteria may also have contributed to the stability of the gastrointestinal microflora in humans. These bacterially derived antimicrobial peptide were first recognized over 80 years ago, when bacteriocin mediated inhibition was observed between two strains of *Escherichia coli*. A variety of different bacteriocins are produced by Gram negative species such as (i) the colicins produced by *E. coli*, microcins produced by enterobacteria [2] or pyocins, which are produced by *Pseudomonas aeruginosa* [3]. Although some Gram negative antimicrobials may ultimately have clinical applications thus far studies have been limited to the use of colicins E1 and N to inhibit other *E. coli* strains which cause post-weaning diarrhea and edema disease in pigs [4]. Gram negative bacteriocins, in general, have narrow spectrums, specifically inhibiting closely related species, and so their potential as therapeutic agents is markedly less than that of their Gram positive counterparts.

Gram positive bacteriocins have been subdivided into as many as five different classes. However, more recently Cotter *et al.* [5] have proposed a re-classification of bacteriocins into two groups. As was the case with previous schemes, the lantibiotics (i.e. post-translationally modified lanthionine-containing peptides) are classified as Class I bacteriocins and Class II consists of the non-lantibiotic peptides. This class has been further subdivided into four subgroups, IIa-d. Class IIa, the Pediocin-like peptides, are generally very active against *Listeria* and are the best characterised of the four subgroups. Class IIb bacteriocins are two peptide bacteriocins, class IIc are cyclic peptides and class IId are linear non-pediocin peptides [5-6].

LANTIBIOTICS – A CHEMICAL PERSPECTIVE

The lanthionine bridges, Lan (lanthionine) and MeLan (β-methyllanthionine), that give the lantibiotics their name are formed through a two-step post-translational modification. The first step involves the enzyme-catalysed dehydration of some, but not all, serine and threonine residues to Dha (dehydroalanine) and Dhb (dehydrobutyrine), respectively. These dehydrated amino acids undergo an intramolecular Michael addition with neighbouring cysteine residues, resulting in the formation of the thioether bridged di-carboxy-di-amino acids, Lan (from Dha) and MeLan (from Dhb). In general, some Dha and/or Dhb residues may remain in their dehydrated form, although in some instances they may undergo alternate modifications. While the serines and threonines in the lantibiotic prepeptide (i.e. unmodified peptide) are all in a levorotatory conformation, both Lan and MeLan are found in the meso-configuration (i.e., DL) with the cysteine-

*Address correspondence to this author at Department of Microbiology, University College Cork, Cork, Ireland; Tel: +353 21 4901373; Fax: +353 21 4903101; E-mail: c.hill@ucc.ie

1389-5575/07 $50.00+.00

derived 'half' present as a L-isomer and the serine/threonine-derived 'half' in the D configuration; i.e. the formation of these thioether bonds inverts the chirality of the α-carbon of serine and threonine residues from the L- to D- configuration [7-8]. These reactions, common to all lantibiotics, thus result in the creation of modified peptides that differ dramatically from those predicted by the corresponding gene sequences, they bring about the creation of residues other than the 20 standard amino acids and result in the formation of an α carbon with a D chiral centre. In cinnamycin an analogous reaction would seem to occur involving the addition of the amino group of a lysine residue to a Dha resulting in the formation of lysinoalanine (Lal) [9]. While Lan, MeLan, Dha and Dhb are by far the most commonly modified residues found in lantibiotics, other less commonly encountered residues include the aforementioned lysinoalanine, lanthionine sulfoxide (oxidized C-terminal MeLan), D-alanine (hydrogenation of Dha), allo-isoleucine, Me_2N-Ala (bis-methylation at Ala1), erythro-3-hydroxyaspartate, 2-oxopropionate (addition of H_2O to an N-terminal Dha followed by spontaneous deamination), 2-oxobutyrate (as previous but Dhb is the N-terminally located residue), 2-hydroxypropionate (occurs when 2-oxopropionate is reduced), S-(2-aminovinyl)-D-cysteine (Cys residue at C-terminal is oxidized and decarboxylated before addition to Dha) and S-(2-aminovinyl)-3-methyl-D-cysteine (as before but addition is to Dhb) [10-11] (Fig. **1**).

As a consequence of the presence of these modified residues, the elucidation of the primary and secondary structures of lantibiotics can be difficult. While Dha and Dhb can remain stable within the peptide chain, their presence can prevent amino acid sequencing by Edman degradation [9, 12-14] as N-terminally located Dha and Dhb residues, either naturally or as a consequence of exposure during Edman degradation, undergo spontaneous deamination through the addition of water molecules resulting in the generation of the sequence blocking groups 2-oxypropionate (2-op) and 2-oxobutyrate (2-ob). This is the reason that the structure of nisin, the prototypic lantibiotic first discovered in the 1940s, was not elucidated until 1971 [13]. In the intervening years relatively few additional lantibiotics have had their structure elucidated. Developments have occurred that facilitate primary sequencing of the peptides through derivatization of dehydrated and thiol-containing residues [15] but this strategy fails to reveal any information with regard to the specific bridging patterns of lanthionine linkages. While strategies have been described which allow the determination of which dehydrated residues are ultimately involved in bridge formation [16], additional NMR-based methodologies have been required to determine bridging patterns definitively. While structural determination is difficult, a conclusive prediction of lantibiotic structures based on primary sequence alone is impossible. While serines and threonines can be dehydrated and subsequently be involved in bridge formation, unmodified serine and threonine residues are found in many lantibiotics while unmodified cysteines have also been identified (e.g., sublancin 168; [17]). Although a pattern observed among type-A lantibiotics (for definition see below) indicates that the cysteine contributing to Lan or MeLan formation is normally located on the C-terminal side of the diamino acid, this arrangement is not uniformly true for the type-B lantibiotics [9, 18]. Thus if one were to consider a hypothetical unmodified lantibiotic possessing the amino acids Thr-Ser-Asn-Thr-

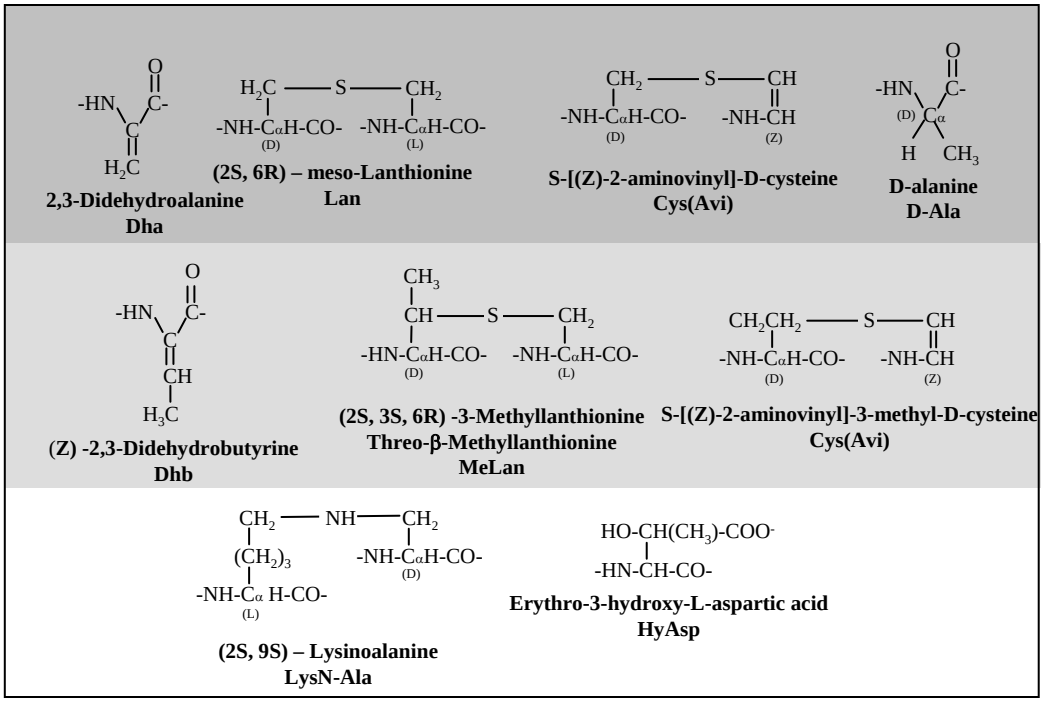

Fig. (1). Modified residues found in lantibiotic.

Cys the number of possible outcomes based on the reactions we have described thus far is at least twenty six (Fig. **2**). Although it has to be noted that the number of potential combinations is in reality limited by the impact of adjacent residues on the modification of amino acids [19] and the requirement for additional modification enzymes in order for some of these modifications to occur [19-23], nonetheless when one considers that lantibiotic propeptides (unmodified structural component) are generally 18 (cinnamycin-like lantibiotics) to 35 (lactocin S, sublancin) amino acids in length, and the fact that these peptides contain between 24 and 47% cysteine, serine and threonine content, then the number of possible combinations becomes staggering.

LANTIBIOTIC CLASSIFICATION

Lantibiotics have previously been subdivided into type-A and type-B peptides on the basis of chemical and structural features [9, 24]. Type A lantibiotics were originally identified on the basis of their elongated structure and their ability to form pores in the cell membrane of target cells, while type B lantibiotics have a globular structure and generally act through the inhibition of enzymatic reactions. It is important to note that not all lanthionine-containing peptides fall neatly into these categories. Some lantibiotics, such as the cinnamycin-like lantibiotics, inhibit the activity of specific medically-significant human enzymes [25], while others such as nisin possesses both type-A and type-B activities. The majority of the two-peptide lantibiotics, which are the subject of this review, are likely to fall into this latter category of possessing dual mechanisms of action, although the situation is obviously further complicated by the involvement of two lanthionine-containing peptides. For these reasons a newer nomenclature has been proposed whereby the lantibiotics are subdivided into 11 groups based on the homology of aligned unmodified structural peptides. These 11 groups (nisin, epidermin, pep5, streptin, lacticin 481, LtnA2, mersacidin, cytolysin, lactocin S, cinnamycin and sublancin) were named after the prototypic lantibiotic in each case [23]. Of the seven two-peptide lantibiotics identified to date, in six instances (lacticin 3147 produced by *Lactococcus lactis* DPC3147 [26]; staphylococcin C55 produced by *Staphylococcus aureus* C55 [27]; plantaricin W produced by *Lactobacillus plantarum* [28]; Smb produced by *Streptococcus mutans* GS5 [29]; BhtA produced by *Streptococcus rattus* BHT [30]; and haloduracin produced by *Bacillus halodurans*, [31] the individual peptides are examples of mersacidin-like and LtnA2-like peptides, respectively, while the two cytolysin (produced by *Enterococcus faecalis*) peptides are highly similar and represent the sole members of the cytolysin-like peptides [32] (Fig. **3**).

MODE OF ACTION OF LANTIBIOTICS - GENERAL

Lantibiotics frequently have a wide spectrum of activity, although activity against Gram negative bacteria is generally limited as a consequence of the protective effect of the associated outer membrane. As noted above, lantibiotics were originally mooted to function *via* either of two distinct modes of action. Type A lantibiotics were associated with the formation of pores in the bacterial cytoplasmic membrane which led to rapid cell death following the loss of essential low molecular mass constituents [33]. Until the late 1990s, type A lantibiotics were thought to function independently of a receptor molecule. During the intervening years it has been shown that a number of these peptides use an essential intermediate in cell wall biosynthesis (lipid II; undecaprenyl-pyrophosphoryl-MurNAC(pentapeptide)-GlcNAc) as a re

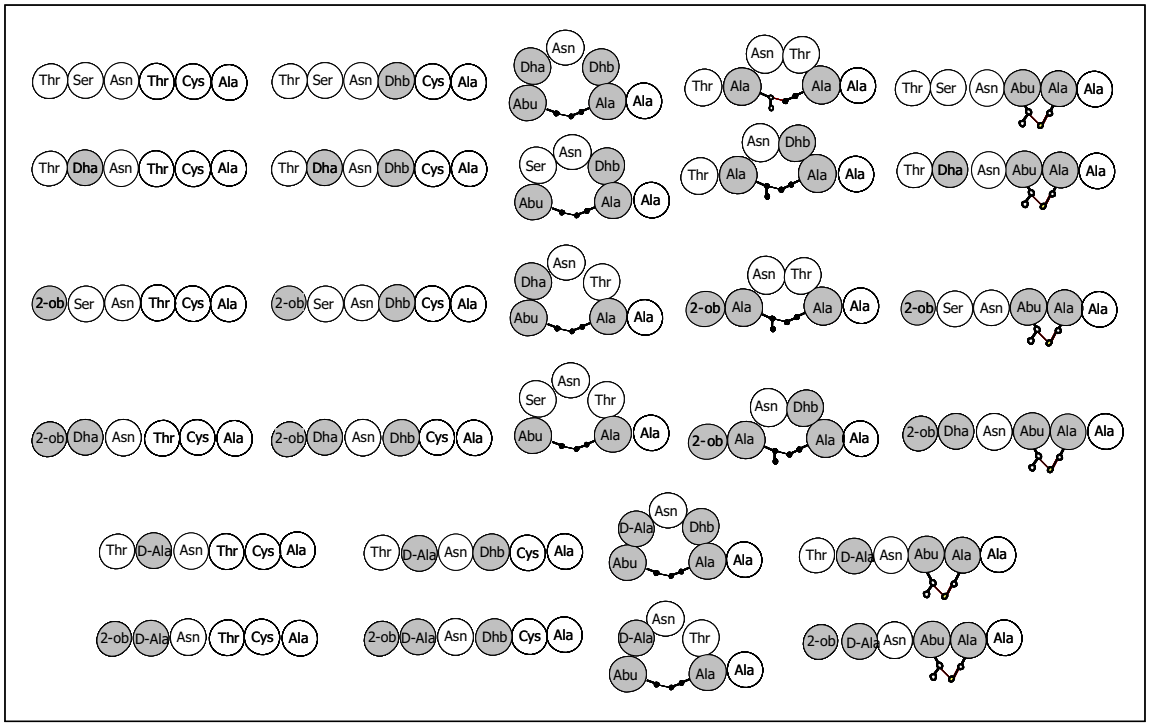

Fig. (2). Possible outcomes following post-translational modification of the hexapeptide TSNTCA (modified residues in grey).

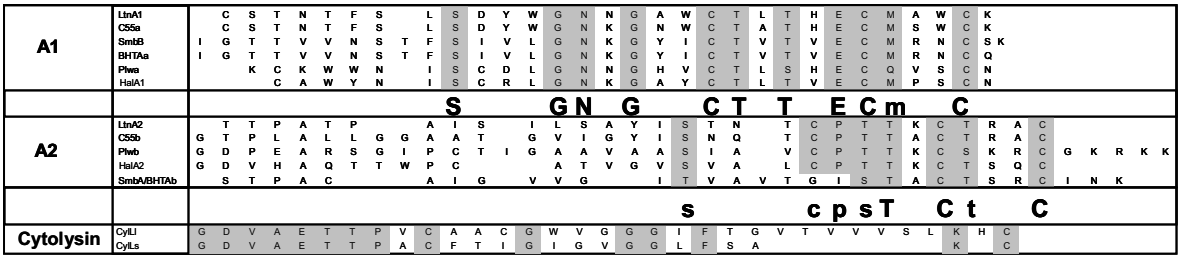

Fig. (3). Alignment of the unmodified propeptide sequences of two-peptide lantibiotics. Highly conserved residues are boxed in grey. Fully conserved residues are indicated by an underlying higher case letter while residues conserved in all but one case are indicated by an underlying lower case letter.

ceptor. Furthermore, the binding of lipid II results in its sequesterization, thus impacting on the integrity of the cell wall [34-35]. Hasper *et al*. (2006) [35] hypothesise that by sequestering lipid II from the site of cell division, thus removing the essential precursor of cell wall biosynthesis and ensuring that lipid II is unable to co-localize with peptidoglycan production, cell wall development can not occur, ultimately leading to the death of the bacterial target. Numerous experiments have confirmed this dual mechanism of action model; it is apparent that in the absence of lipid II, much higher concentrations of nisin are required for pore formation [36], it has been established that the N-terminus of nisin, in particular rings A and B, is responsible for the specific binding of lipid II [37-38], that its C-terminus is of key importance for pore-formation and that the region around rings C and D is responsible for the conformational flexibility required to link the two functions [39]. It is significant that the nisin (and in all likelihood epidermin) -like peptides bind lipid II at a site (N-Ac-Muramyl-pentapeptide) which is distinct from that bound by vancomycin (N-acyl-D-ala-D-ala), which for many years has been the drug of last resort when treating Gram positive infections. As a consequence, the activity of nisin against vancomycin resistant enterococci is not impaired [40]. The antimicrobial consequence of lipid II binding is the reason why lantibiotics such as epidermin, which are only able to form pores in thin model membranes and specific target cells, retain high level killing [35]. It has recently been established that a member of the lacticin-481 lantibiotics, plantaricin C, produced by *Lactobacillus plantarum* LL441, is also a lipid II interacting peptide and also inhibits cell wall biosynthesis. Like epidermin, plantaricin C cannot form pores in $C_{18:1}$ liposomes [41], again indicating an inability to traverse membranes of this size. Mersacidin is incapable of pore-formation; functioning solely through the lipid II-binding mediated inhibition of peptidoglycan synthesis [42]. Mersacidin binds lipid II at the disaccharide-pyrophosphate moiety, a target-binding site that is not currently utilised by any commercially available antibacterial drug. This binding blocks transglycosylation, so peptidoglycan sugar chains are not polymerised [11]. Mersacidin is unable to bind to lipid I, and has a much lower affinity for lipid II than nisin. The negatively charged glutamate at position 17 has been hypothesized to be important for Lipid II binding as its replacement with an alanine abolishes its ability to function [37].

Cinnamycin and cinnamycin-like peptides (duramycin, duramycin B, duramycin C and ancovenin) also act on target strains by disrupting enzymatic function, including medically significant human enzymes such as phospholipase A2 (acted on by the duramycins) and angiotensin converting enzyme (ACE) (acted on by ancovenin). Cinnamycin itself is capable of inhibiting phospholipase A2 and ACE [43-45]. The receptor for cinnamycin-binding has been identified as phosphatidylethanolamine (PE) which resides in the inner layer of the plasma membrane [46-47].

LANTIBIOTICS – A GENERAL MEDICAL PERSPECTIVE

A number of lantibiotics have demonstrated significant potential as antimicrobials for medical applications. Nisin is the most extensively characterised of all the lantibiotics. It has a long history of use in the food industry, has been approved for use as a food preservative/additive and is employed in over 50 countries worldwide [48]. However, this peptide also exhibits activity against clinically significant bacteria such as methicillin resistant *Staphylococcus aureus* (MRSA), vancomycin resistant enterococci [49-50] and *Clostridium difficile* [51], as well as being potentially of use for the treatment of peptic ulcers due to its ability (a rarity for lantibiotics) to eliminate the gram negative *Helicobacter pylori* [52]. It may also have veterinary applications based on its ability to treat bovine mastitis [53]. More recently it has been demonstrated that nisin may also be utilised as an effective contraceptive in rat, rabbit and monkey models. It completely impedes sperm motility and so blocks conception, but it also has the advantage of being an antimicrobial and may also help in the prevention of the spread of sexually transmitted diseases [54-55].

A number of other lantibiotics have shown efficacy against clinically significant Gram positive bacteria. Mersacidin is a 20-amino-acid peptide produced by *Bacillus subtilis* HIL-Y85, 54728 It is the smallest lantibiotic studied to date at 1825Da and contains three methyllanthionine residues, one S-aminovinyl-2-methylcysteine and a single dehydroalanine. It is of particular interest with respect to this review in that it and the A1/α component of the majority of two-peptide lantibiotics are members of the mersacidin-like subgroup. This lantibiotic has attracted significant interest due to its wide spectrum of activity, including activity against MRSA [18]. Kruszewska *et al.* [56] have investigated the efficacy of mersacidin against MRSA using a murine model. Two distinct dosage levels were employed in this study- low dosage ($3 \times 10^{-2} - 10^{-4}$cfu) and high dosage ($3 \times 10^{-6}$-$10^{-8}$ cfu). Hydrocortisone, an immunosuppressant, was

administered prior to intranasal application of the lower MRSA inoculum. This resulted in a local intranasal infection. 1.66mg/kg/treatment of mersacidin was administered through the intranasal route, twice daily over three days, to effectively clear the MRSA infection [56].

The epidermin lantibiotic is a 21 amino acid, tetracyclic peptide, containing a single MeLan and two Lan, produced by *Staphylococcus epidermidis* Tü3298 [11]. A number of natural variants of epidermin have been reported. Gallidermin (produced by a *Staphylococcus gallinarum*) differs from epidermin by a single amino acid at position 6 (Ile6Leu) while epidermin' also differs at this location in addition to an Ile1Val change. Gallidermin was observed to have more activity than epidermin against Gram positive pathogens such as *S. aureus* and *Propionibacterium acne* [14]. Nascimento *et al*. [57] have utilised epidermin against a range of MRSA strains with promising results. It was established that epidermin was an effective agent for the control of MRSA, to the extent that future therapeutic use, either in the treatment or prophylaxis of bacterial infection, would appear to be a viable option [57]. It has also been recently revealed that epidermin is capable of preventing *Staphylococcus epidermidis* adhering to catheters. The adhesion of *S. epidermidis* to catheters in hospitals is a major concern as patients who have indwelling central venous catheters are at increased risk of developing bacterial infections caused by coagulase negative *Staphylococci* (CNS). The treatment of catheters either pre or post bacterial colonisation with epidermin has been shown to be extremely effective [58]. Pep5, a 34 amino acid lanthionine peptide produced by *S. epidermidis* 5, was also investigated and was able to impede the growth of *S. aureus* strains tested. Mutacin 1140, an epidermin-like peptide, is a licensed and patented antimicrobial peptide produced by *Strep. mutans*. This lantibiotic peptide has a very expansive range of kill, eradicating essentially all Gram positive bacteria tested to date as well as some clinically significant Gram-negative strains. Medically important bacterial strains, such as those responsible for gastric ulcers, pneumonia, listeriosis and "strep" throat are all eradicated by mutacin 1140 [59; www.oragenics.com]. From an oral microbiology perspective, another interesting development has been the use of *Streptococcus salivarius* K12, a producer of two lantibiotics, salivaricin A2 and salavaricin B [60], to control the growth of oral bacteria responsible for bad breath. Studies have shown that individuals with low levels of *Streptococcus salivarius* K12 suffered from chronic bad breath, whereas persons with high levels of the bacterium had healthy breath. A related product aids in the support of a healthy bacterial flora in the system, particularly after antibiotic use. This product also utilizes *Streptococcus salivarius* K12, but in conjunction with three lactic acid bacterial strains [61-62]. Recently, two Salvaricin A producing strains (*S. salivarius* 20P3 and 5) were incorporated into a milk drink given to children, results indicated that the probiotic strains were capable of colonization of and persistence in the oral cavity. The data generated in this study infers that consumption of this probiotic is inhibitory to *S. pyogenes* [63]. *S. salivarius* has also been shown to be inhibitory to the pathogen *P. acne*, the causative agent of acne of the skin and so may be used in its treatment [64].

TWO-PEPTIDE LANTIBIOTICS

While many of the lantibiotics were first identified more than fifteen years ago, the identification of two-peptide lantibiotics has been a more recent, and rare, event. As the name implies, two peptide lantibiotics require the presence and expression of two structural genes, the individual products of which are themselves inactive (or display little activity) but when combined exhibit significant levels of broad-range antimicrobial activity (Table **1**). The individual peptides are LtnA1 and LtnA2 (lacticin 3147), $CylL_L$ and $CylL_S$ (cytolysin), C55α and C55β (staphylococcin C55), Plwα and Plwβ (plantaricin W), SmbA and SmbB (Smb), BHT-Aα and BHT-Aβ (BhtA) and Halα and Halβ (haloduracin). For consistency, we will refer to α and β peptides in all instances.

Lacticin 3147

An Irish kefir grain was the original source of the food grade strain *Lactococcus lactis* subsp. *lactis* DPC3147, the producer of the two-peptide lantibiotic, lacticin 3147. This potent antimicrobial displays activity at single nanomolar concentrations and is active against a wide spectrum of gram-positive bacteria (Table **1**; [65]). The 60.2 kb self-transmissible plasmid, pMRC01, carries ten genes that are responsible for lacticin production (*ltnA1*and *A2*), modification (*ltnM1*, *ltnM2* and *ltnJ)*, regulation (*ltnR*), transport (*ltnT*) and immunity (*ltnIFE*). The structural genes *ltnA1* and *ltnA2* encode peptides with molecular masses, following modification and leader cleavage, of 3305 and 2847 Da, respectively (Fig. **2**). The structure of both peptides has been elucidated by multidimensional NMR spectroscopy [16]. This was the first occasion upon which the structure of the peptides of a two-peptide lantibiotic was elucidated (Fig. **4**) and established definitively that post-translational modification results in the presence of Lan, MeLan, Dhb, 2-oxobutyrate and, most unusually, D-alanine in the lacticin 3147 peptides. The presence of D-amino acids in ribosomally-synthesised peptides and proteins is exceedingly rare in nature as most D-amino acid containing proteins result by non-ribosomal means through multienzymic complexes. In prokaryotic biology the presence of D-amino acids in ribosomally synthesised peptides has until recently [66] been associated specifically with two lantibiotics; i.e. lacticin 3147 and lactocin S (a one peptide lantibiotic). With the exception of these residues, all D-amino acids in gene-encoded peptides result from the stereoinversion of an amino acid from the L- to its D-isoform. In stark contrast to this sequencing of the *ltnA1* and *ltnA2* genes has revealed the presence of L-serine codons at the position corresponding to the D-alanine residues in the mature peptides [16, 24]. The L-serine is dehydrated to generate Dha, which is then the subject of stereospecific hydrogenation to produce D-alanine [6]. Thus remarkably there is a change in both the identity and chirality of the amino acid involved. While a definitive role for the D-alanine residues in lacticin 3147 will only become apparent through structural analysis of peptides in an active conformation, site-directed mutagenesis has revealed that they are essential with respect to the optimal activity and/or production of the peptides. It was found that replacement of D-alanines with non-chiral amino acids (glycine, Dha or Dhb) had a less dramatic impact than that which resulted when L-amino acids (L-alanine or L-valine) were in-

Table 1. Spectrum of Inhibition of the Two Component Lantibiotics

Lantibiotic	Known Sensitive Strains
Lacticin 3147	*Lactococcus lactis, Enterococcus faecalis, Lactobacillus gasseri, Staphylococcus aureus, Pediococcus pentasacaeus, Micrococcus luteus, Listeria monocytogenes.*
Staphylococcin C55	*Lactococcus lactis, Lactobacillus paracasei, Staphylococcus aureus DPC5245, Listeria monocytogenes, Bacillus subtilis, Micrococcus luteus, Leuconostoc oenes.*
Haloduracin	*Lactococcus lactis, Listeria monocytogenes, Enterococcus faecalis, Streptococcus mutans, Pediococcus pentosacaeus, Bacillus cereus, Lactobacillus bulgaricus.*
Plantaricin W	*Lactococcus lactis, Lactobacillus spp., Oenococcus oenos, Leuconostoc mesenteroides, Bacillus subtilis, Listeria monocytogenes, Staphylococcus aureus.*
Smb	*Streptococcus mutans, Streptococcus oralis, Streptococcus sanguinis, Streptococcus pneumoniae, Enterococcus faecalis, Staphylococcus epidermidis.*
BHT-A	*Micrococcus luteus, Lactococcus lactis, Streptococcus pyogenes, Streptococcus mutans, Streptococcus sobrinus.*
Cytolysin	All gram positive bacteria tested and also Eukaryotic cells

corporated. The presence of D-alanine residues within a putative α-helical region in Ltnβ may indicate that they play a role in pore-formation. Three enzymes (LtnM1, LtnM2 and LtnI) have been associated with the post-translational modification of lacticin 3147. The LanM lanthionine synthetases have been shown to catalyze the dehydration and cyclization reactions that bring about Lan and MeLan formation. The biosynthesis of lacticin 3147 is dependent of two such enzymes, LtnM1 and LtnM2, which are specific for the modification of Ltnα and Ltnβ respectively [67]. The production of an active antimicrobial is also dependent on catalysis by LtnJ, a dehydrgenase-like enzyme responsible for Dha to D-alanine conversion [6]. Although LtnJ is the only enzyme known to be capable of performing this function, it is interesting to note that a homologous protein, SacJ, may be involved in biosynthesis of the closely related two peptide lantibiotic, staphylococcin C55.

A number of studies have been carried out to investigate the mode of action of lacticin 3147. Significantly, the lanthionine-bridging pattern of Ltnα was found to be remarkably similar to that of the single peptide, lipid II-binding lantibiotic, mersacidin, which functions by inhibiting cell wall biosynthesis. It was ultimately established that Ltnα also has the ability to bind lipid II [41] and this binding is thought to be mediated by a putative lipid II-binding domain (CTxTxEC) common to all mersacidin/Ltnα like peptides [68]. Following the interaction of Ltnα with the cell membrane it would appear that it undergoes a conformational change. It is possible that this change results in an Ltnβ-binding site becoming available. Although this conformational change also occurs in membrane environments lacking lipid II, it is at a much lower frequency. The presence of Ltnβ enhances the affinity of Ltnα for lipid II and allows deeper insertion into the membrane thus enabling Ltnβ to assume a transbilayer orientation. This allows rapid release of K^+ and phosphate ions leading to immediate membrane potential dissipation, hydrolysis of intracellular ATP and cell death. The size of the pores formed by lacticin 3147 have been found to be significantly smaller (25%) than those produced by nisin [41]. As a consequence of the homology between the predicted unmodified peptides across all lacticin 3147-like two peptide lantibiotics, it is anticipated that these other lantibiotics will have a similar mechanism of action.

Haloduracin

Bioinformatic analysis revealed that *Bacillus halodurans* C-125 may also produce a lantibiotic. It was established that a significant degree of homology existed between a number of C-125 proteins and those associated with lantibiotic production and immunity in other strains, and the two putative structural peptides (Halα and Halβ) were identified [31, 69]. The relevant region of the C-125 chromosome is 15Kb in length and contains eleven open reading frames. It is believed that these genes are transcribed in three units, but this has yet to be demonstrated experimentally. The first operon is thought to contain six genes (*bh0445-bh0450*) with roles in lantibiotic immunity. Unusually, there appears to be two sets of immunity genes (each set encoding an ABC transporter unit) and the reason for this is unknown. The next operon carries three genes (*bh0451-bh0453*). The product of *bh0451* (*halT*) is believed to play a similar role to typical LanT proteins i.e. cleavage of the leader region and export of the mature peptide. The product of *bh0452* (*halM2*) is also carried on this putative transcriptional unit and is believed to be responsible for the modification of one of the structural peptides i.e. *halβ* (encoded by *bh0453*). Located on the final operon (*bh0454-bh0455*) are the genes predicted to encode the *halα* structural peptide and *halM1*, its corresponding modification enzyme [69].

Extensive studies carried out by McClerren *et al.* revealed interesting observations about the Halα and Halβ peptides. Results indicated that HalA1 undergoes three dehydrations, leaving one unmodified Ser and interestingly contains a disulphide bridge, thus representing one of only three examples of such a structure in a natural lantibiotic (after plantaricin W and sublancin 168). It was noted that Halβ also contains a single unmodified Ser/Thr residue. Analysis of the LanM enzymes revealed that each enzyme could only act specifically on its corresponding structural peptide and, significantly, that the activity of this enzymes could be reconstituted *in vitro* [31].

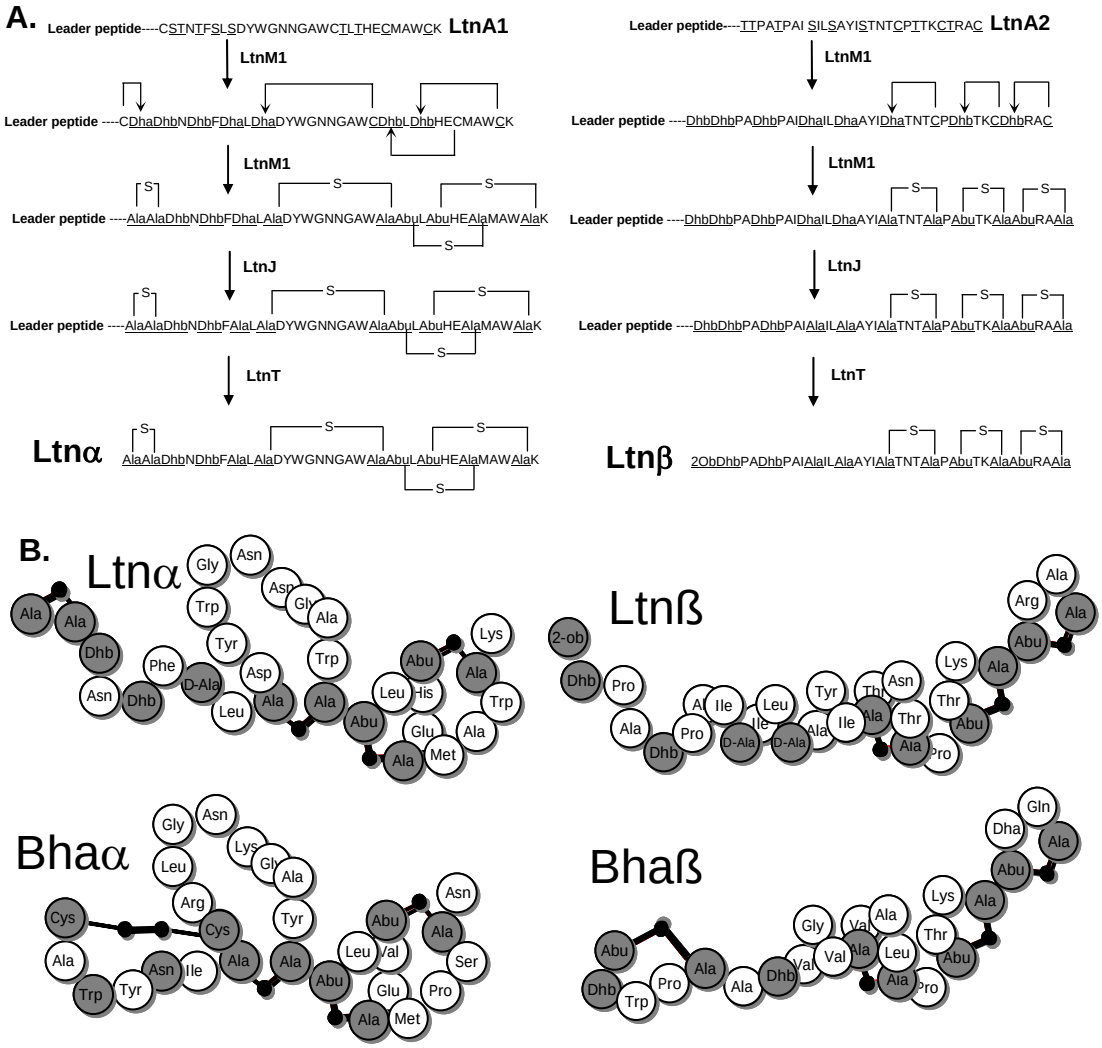

Fig. (4). A. Modification of propeptides of the lacticin 3147 peptides. Ala-S-Ala, lanthionine; Ala-S-Abu, Abu-S-Ala, β-methyllanthionine. B. Structure of lacticin 3147 peptides (Ltnαβ) and the predicted structure of the haloduracin peptides (Bhaαβ) as representative two peptide lantibiotic structures.

Staphylococcin C55

In 1970, Dajani *et al.* [70] reported the production of an antimicrobial substance by *Staphylococcus aureus* C55. Further analysis of the antimicrobial by Navaratna *et al.* [71] revealed the antimicrobial substance was a two-peptide lantibiotic. It was established that when the two peptides (C55α and C55β) were combined in equimolar concentrations they functioned synergistically to inhibit a wide variety of Gram-positive bacteria. The genes responsible for production of staphylococcin C55 are carried on a 32kb plasmid and curing of this plasmid at elevated temperatures leads to the loss of antimicrobial activity. The genetic determinants for staphylococcin C55 have also been identified in other *S. aureus* plasmids; e.g. a 37Kb plasmid located in *S. aureus* U0007 [72] and a 38Kb plasmid in *S. aureus* TY4 [73]. The genes involved in C55 production are located between two insertion sequences (IS257). *sacαA-βA-M1-T-M2-orf45-46-47-48* are transcribed together in a single operon while *orf38* and *39* are divergently expressed [73]. The individual staphylococcin C55 peptides, C55α and β, closely resemble the corresponding peptides of lacticin 3147. The unmodified α peptides exhibit 86% identity, differing with respect to only four amino acids i.e. N15K, A17N, L21A and A27S (the relevant Ltnα residue being listed first in each case). The β peptides exhibit 55% identity and show highest homology over a 16 amino acid region at the C-terminal end. Interestingly, cross complementation studies in which Ltnα -C55β and Ltnβ-C55α combinations were tested revealed that high level activity was apparent in both cases. This in itself is a testament to the high degree of relatedness between the two sets of structural peptides [74].

Plantaricin W

Plantaricin W (Plw) is the product of *Lactobacillus plantarum* LMG 2379. This two-peptide bacteriocin was found to inhibit a wide range of gram positive bacteria (Table **1**). Plwα was found to contain 29 residues in addition to the 30 amino acid leader region and is also unusual in that in con-

tains a disulphide bridge while Plwβ contains 32 amino acids. Reverse phase chromatography successfully separated both peptides. Little or no activity was observed when the fractions were tested individually but when they were combined, a high level of activity was detected, indicating that both peptides are required for optimal activity. It was established that a 1:1 ratio of the two peptides resulted in optimal killing of the sensitive strain *Lactobacillus sakei* NCDO. Investigations established that the majority of the cysteines, serines and threonines in the peptides underwent post-translational modification [28]. Like the closely related Halα peptide, it has been suggested that Plwα is unusual in that it contains a disulphide bridge [28]. It has also been suggested that a free cysteine is located in Plwβ, however, as this contrasts with the predicted Halβ structure [31], the form that this residue takes in the final, modified, peptide may need to be reassessed.

Smb

Some *Streptococcus mutans* strains are known to produce antimicrobial substances known as mutacins. Up until recently four mutacins had been identified; i.e the lantibiotic mutacins I, II and III and the non-lantibiotic dipeptide mutacin IV. Recently a novel two-peptide lanthionine-containing mutacin has been identified and designated Smb [29]. This lantibiotic displays a wide spectrum of activity against oral streptococci, including *Streptococcus mutans*, which may have significant implications for complex oral biofilms in particular and the treatment of bacterial infections in general [75]. The gene cluster responsible for the biosynthesis of Smb consists of seven genes, in the order *smbM1, -T, - F, -M2, -G, -A, -B*, which form a single operon spanning a 9.5Kb region on the chromosome (Fig. **5**). *smbA* and *smbB* encode the Smb structural peptides (the unmodified propeptides are 30 and 32 amino acids in size, respectively). Production of Smb is dependant on induction by competence stimulating peptide (CSP) *via* a quorum sensing mechanism. When *comC*, the gene encoding CSP, is mutated, bacteriocin production ceases unless CSP is added exogenously, in which case bacteriocin production is restored. To confirm that both peptides were required for optimal activity a variety of mutants were generated. Disruption of *smbA* or *smbB* resulted in a pronounced reduction in antimicrobial activity. It can therefore be deduced that both peptides are required for optimal activity and act in a synergistic manner [29]. Further proof of this was more recently obtained by Peterson *et al.* [75] in that both peptides were purified to homogeneity and failed to display any activity when they were assayed individually but activity was restored when the peptides were present in concert. This study also showed that the first amino acid of the Smbβ peptide is not a Gly as reported previously [29], but rather an Ile, making the lantibiotic more similar to BHT than previously suspected.

BHT

Another oral *Streptococcus*, *Streptococcus rattus* BHT, is also the producer of a two-peptide lantibiotic. *S. rattus* BHT was isolated from a human source and principally colonises the surface of the tooth. However, it is not believed to be one of the main contributors to dental caries due to its low numbers in plaque. Investigations by Hyink *et al.* [30] revealed that *S. rattus* BHT in fact produces both a class I and a class II bacteriocin (BHT-A and BHT-B respectively). It was established that BHT-A is a two peptide lantibiotic that is a variant of Smb, as evidenced by the fact that the two operons share 95% identity. The eight genes are all transcribed in the same direction as follows BHT-R, -M1, -F, -G, -M2, -T, -Aβ, and -Aα (Fig. **5**). MALDI-TOF mass spectrometry established that the associated molecular masses were 3375 and 2802 Da for the α and β peptides, respectively. While synergy between the two peptides has yet to be definitively proven, the attenuated bacteriocin activity of BHT-Aα alone indicates that it is extremely likely [30].

Cytolysin

Cytolysin a two peptide lantibiotic produced by *Enterococcus faecalis* encoded on a pathogenicity island or pheromone response plasmids [76] that differs greatly from the

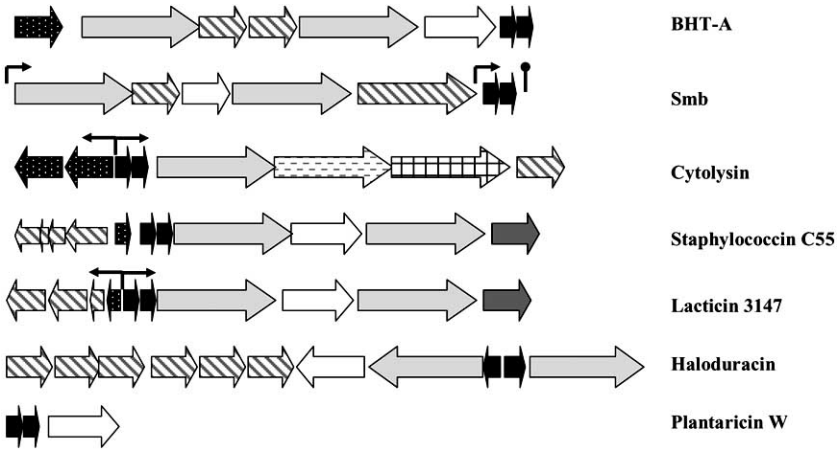

Fig. (5). Biosynthetic gene clusters of the two-peptide lantibiotics. Structural genes (*lanA*) are indicated by black arrows, modification genes are shown as grey (*lanM*) and dark grey (LanJ) arrows. Immunity genes are indicated by diagonal stripes. Genes involved in transport and leader cleavage are shown as white arrows. Regulatory genes are illustrated as white spotted arrows. Arrows with dashed black lines have a secretory function, genes involved in N-terminal cleavage are demonstrated by arrows with black squares. Stem loop terminators and promoters are present as lollipops and line arrows, respectively.

other two-peptide lantibiotics, not only with respect to its amino acid sequence (Fig. **3**) but also by virtue of the being the only lantibiotic with cytotoxic activity. Eight genes, carried on two divergent operon-like structures, are involved and are regulated by the products of two genes, *cylR1* and *cylR2,* through a quorum sensing mechanism [77]. The CylR1/2 system differs from other well known signal transduction systems in that neither of the genes bears significant homology to the two peptide regulator superfamily [78]. This signalling is mediated through the structural peptides themselves. Cytolysin reacts to the presence of target cells through binding of one of the two peptides, $CylL_L$, to the target. This leads to an accumulation of free $CylL_S$ (i.e. the second peptide which in the absence of target cells is bound to $CylL_L$) to exceed threshold levels and express elevated levels of the toxin in reaction to this [76].

$CylL_L$ is 38 amino acids in length and $CylL_S$ is 21 amino acids in length [79]. There is a high degree of similarity between the two prepeptides with 26 highly conserved residues present. Once the leader has been cleaved the N-termini share 10 identical residues however the C-termini do not share this degree of similarity. Both prepeptides are post translationally modified by the product of the *cylM* gene. After modification the peptides are processed and secreted by CylB*;* externalised products have 6 amino acids removed from the N- terminus by ClyA. Finally CylI is responsible for producer self protection from its own bacteriocin [77].

CONSERVED RESIDUES IN STRUCTURAL PEPTIDES

A number of conserved residues exist in the α and β peptides (Fig. **3**). Of the α group, Ltnα, C55α, Halα and Plwα all possess a conserved GA leader cleavage site. A conserved motif, SxxxGNxGxxCTxTxECmxxC, common to all of the α peptides has been identified and within this motif a shorter stretch, GxxxxTxs/tCd/eC(3-10x)C, is shared with all lacticin 481- and mersacidin-like peptides. The β peptides of Halβ, Cyl_L and Cyl_s and Plwβ contain a conserved GD motif that occurs just after the site of leader cleavage. Mutation of conserved residues within a motif common to all β peptides, s/txxxxc**p**s/t**T**xCs/txxC, has been shown to impact severely on Ltn β bioactivity. While the consequences of mutation of the proline and threonine residues (shown in bold) is relatively less severe it should be noted that mutagenesis of the proline residue impacts harshly on peptide:peptide synergism. Complete alanine scanning mutagenesis of lacticin 3147 has revealed the importance of individual amino acids in Ltnα, Ltnβ, and by extension, conserved residues in related two-peptide lantibiotics. It was apparent from the study that of the 59 residues 36 could be altered to alanine and yet retain a significant bioactivity. In Ltnα, there are 13 residues that cannot withstand mutation (F6, S7, S9, W12, N14, W18, C19, T20, L21, T22, E24, C25, W28, C29). Alteration of these amino acids resulted in complete loss of activity, when tested against the sensitive indicator *L. lactis* HP. Ltnβ was, in general, was more amenable to change, since the mutation of only seven amino acids resulted in complete loss of activity (S16, N18, C20, T22, C25, T26, C29). These investigations will greatly aid the rational drug design of these peptides in that although no mutation resulted in increased bioactivity, the exercise demonstrated that residues are amenable to change and there is the possibility of creating a mutation in the future which will be more active, have a greater spectrum of activity or may overcome resistance issues [68]. Furthermore, as a result of this and other [6] studies, a total of 71 lacticin 3147 derived mutants have now been generated. This total surpasses the combined number of all other lantibiotic mutations published to date.

Random mutagenesis of lacticin 3147 peptides has also been carried out and has proved to be an invaluable tool. An error-prone PCR was utilised in order to randomly insert mismatches that may result in the conversion of a native amino acid to a differing amino acid. In this particular study all mutant peptides resulted in reduced or abolished activity and comparison of data generated here with those generated in the site specific peptide engineering study were in close agreement, reinforcing both approaches. Some mutations resulted in a total loss of bioactivity (α:W12C, α:N14I, α:W18C, α:L21F, α:W28R and β:N18K), whereas other simply resulted in a decline in bioactivity (α:D10A, α:A27V, α:A27T, α:C1S, α:S2N). α:C1 and α:S2 are both involved in lanthionine bridge formation at the N terminal, their subsequent alteration to Ser and Asn respectively caused a reduction in bioactivity but not elimination, indicating that this bridge is not requisite for full antimicrobial activity. A number of mutations were also made in the leader regions of the structural genes yielding variable results. Of eight mutants in the leaders of both peptides only two (α:E-14G and α:W-17L) lacked bioactivity [80], which in itself is unusual in that previous studies have illustrated that mutagenising leader regions can have vast implications on production of peptide [81-83].

TWO PEPTIDE LANTIBIOTICS – A MEDICAL PERSPECTIVE

The two peptide lantibiotics are of particular medical interest in that while a number exhibit activity against a broad range of Gram positive pathogens, the staphylococcin C55 genes are consistently associated with the genes for exfoliative toxin B (ET-B) and cytolysin is itself cytotoxic to eukaryotic cells. Here we expand on the medical significance of the two-peptide lantibiotics.

Lacticin 3147 is of particular interest from an applications point of view. To date an expansive list of potential applications has been proposed. Early studies on the lantibiotic revealed its ability to eradicate an impressive list of the so-called superbugs. It was demonstrated that 3147 had activity against methicillin resistant *Staphylococcus aureus* (MRSA), vancomycin resistant *Enterococcus faecalis* [49], penicillin resistant *Pneumococcus*, *Propionibacterium acne* and *Streptococcus mutans,* all of which are significant human pathogens, presenting a multitude of applications for which it may be employed [84]. Experimental studies have verified its effectiveness in the treatment of mastitis in cattle. Teat seals were used either independently or in combination with lacticin 3147, it was observed that after deliberate infection with the pathogen *Streptococcus dysgalactiae*, that 61% of the control quarters were either shedding the organism or had developed clinical mastitis, in comparison with 6% of the treatment teat seals [26]. It may be construed from this that lacticin 3147 may also be effective in the treatment of *S.*

dysgalactiae in humans; this microorganism has been implicated in the occurrence of acute pharyngitis in children [85] and has also been implicated in a case of bacteraemia in a patient with pyomyositis and reactive arthritis [86]. Cases where *S. dysgalactiae* has been diagnosed as the causative agent in vertebral osteomyelitis, an uncommon cause of back pain in adults, where prolonged antibiotic therapy (> 6 weeks) is required to clear infection [87] have also been recorded. From a clinical point of view the strain *Streptococcus agalactiae* is possibly a more significant pathogen as it has long been associated with perinatal morbidity and neonatal mortality, as well as post partum endometritis in women. Lacticin 3147 has been shown to have the ability to inhibit *S. agalactiae* and *S. dysgalactiae* and so may be a prospective agent for the control of these micro-organisms [88]. More recently lacticin 3147 has also been found to be effective against 15 different cariogenic strains, indicating that it may be exploited in the future for the prevention of dental caries [74]. This lantibiotic may be a viable alternative to conventional antibiotics; especially given the recent explosion of gram positive resistant strains, and the distinct advantage that little or no resistance to lacticin 3147 has been observed [89].

It was noted that in all cases, the bacteriocin producing genes of Staphylococcin C55 are linked to the presence of exfoliative toxin B (ET-B), an exotoxin associated with skin infections in humans [71]. These exfoliative toxins are medically significant as they are responsible for the condition known as staphylococcal scalded skin syndrome (SSSS). The clinical features of this syndrome vary in severity, from localised blistering to sloughing off of the skin covering up to 90% of the body. Initially symptoms such as fever, malaise, loss of appetite or lethargy are apparent but are soon followed by an erythematous rash which subsequently develops into blisters. The occurrence of SSSS in immunocompetent adults is extremely rare, but intradermal injection of purified ET into the forearm of a healthy adult rarely results in SSSS in immunocompetent adults but does result in the development of blisters in healthy subjects. Two serotypes of these staphylococcal toxins exist; ET-B and ET-A, and only 5% of clinical *S. aureus* strains produce either one or both of these toxins. The toxins are extremely species specific, targeting humans, mice, monkeys and hamsters but not rabbits, dogs or rats. Staphylococcal toxins have also been linked to the development of other perplexing conditions; one of the more noteworthy is sudden infant death syndrome (SIDS), *S. aureus* being the most frequently isolated strain from 2-4 month old infants, which are a high risk group for developing SIDS. There is a higher prevalence of SIDS in the winter months, and studies on ferrets concurrently infected with *Staphylococcus* and the influenza virus, led to a drastic increase in production of staphylococcal enterotoxins (SE), perhaps explaining the predominance of SIDS in this time period [90-92]. Aside from the production of ET, there is the possibility that the production of an antimicrobial confers a competitive edge on the producing strain by eliminating other skin microflora with whom they may contend for vital space and nutrients.

The contribution of cytolysin to *Enterococcus faecalis* virulence in a number of medical conditions such as enterococcal endocarditis [93] and endophthalmitis, a sight threatening condition that occurs as a complication of surgery or a penetrative eye injury has been noted. *Enterococcus faecalis* has been reported to be the primary infective agent in endophthalmitis post glaucoma surgery. The prognosis for endophthalmitis is bleak with loss of vision or even enucleation becoming necessary [76]. Further studies in animal disease models established that cytolytic *E. faecalis* not only causes more fulminant disease but also results in the disease becoming insensitive to therapeutic intercession.

DISCUSSION

A logical question is whether two peptide lantibiotics have any advantage over their one peptide counterparts? One possible answer is that the presence of a second peptide results in enhanced antimicrobial activity. Mersacidin is a one-peptide bacteriocin from the same lantibiotic subgroup as the A1/α peptide of the majority of two peptide lantibiotics. It operates by binding to lipid II and inhibiting cell wall biosynthesis. The related peptide Ltnα also possesses this ability, but in addition it can function synergistically with Ltnβ to form pores in the cytoplasmic membrane of sensitive cells leading to a mass efflux of intracellular ions and rapid cell death. Therefore any cells that could potentially survive the action of the α peptide ultimately killed by the β peptide. At present this theory remains speculative, as little is known about the kinetics of cell death due to bacteriocins and it would also be difficult to compare the kinetics of one peptide to two peptide induced death. It is also possible to dispute this if we take a closer look at the single peptide bacteriocin, nisin and the two-peptide bacteriocin, lacticin 3147. Nisin functions by binding to lipid II in the same manner as lacticin 3147 and forming pores, causing leakage of cell constituents. However, the pores formed by nisin are approximately 2nm in diameter whereas the pores formed by lacticin 3147 are much smaller, with a diameter of 0.6nm. Thus it would be logical to assume that nisin has the potential to more rapidly dissipate the membrane potential of a target strain and bring about a more rapid cell death. Another alternative is that two-peptide lantibiotics differ with respect to their target range. Here again comparison of nisin and lacticin 3147 would seem to argue against this, in that both lantibiotics are produced by *Lactococcus lactis* from the same ecological niches and have similarly broad ranges of activity. Also, while our experience suggests that the frequency of resistance to lacticin 3147 is reduced compared to resistance to nisin, we have not gathered the data necessary to unequivocally support this possibility .

An alternative view may be that nisin and lacticin 3147 simply represent different lantibiotic-types, the actions of which have evolved in a convergent manner. This model is supported by the fact that some type A peptides, including epidermin, are not capable of pore formation in all target strains. Similarly, lacticin 481- and mersacidin-like non two-peptide lantibiotics (plantaricin C and mersacidin, respectively) function primarily through lipid II binding. In the case of the mersacidin-like peptides, gene duplication, rather than evolution of a single structural peptide, may have occurred to facilitate the added ability to bring about pore formation. Although it would have to have been an ancient duplication event, given that for the Ltnα and β peptides only

share 17% identity, there are some indications that such duplication events have recently taken place in lacticin 481-like peptides. The ruminococcin A producer, *Ruminococcus gnavus*, possesses three copies of the structural gene, *rumA*. When homology studies were carried out on these three copies, >95% identity was observed at the nucleotide level. These particular genes were also found in *R. gnavus* E1, *R. hansenii* and *Clostridium nexile*. In all of these strains the lantibiotic-associated genes have a low G+C content (32-34%) which does not correspond with the average G+C content of the entire genome (38-43%), indicating that perhaps horizontal gene transfer has occurred recently from a bacterium with a lower G+C content [94] . Ruminococcin A is not the sole example of a lantibiotic which possesses multiple copies of a structural gene. This is also the case for macedocin, a food grade lantibiotic produced by *Streptococcus macedonicus* ACA-DC198 which possesses two copies of its structural gene. This particular strain was isolated from Greek Kasseri cheese and inhibits a broad range of lactic acid bacteria, as well as more industrially significant strains such as food spoilage and pathogenic bacteria. N-terminal sequencing revealed that 22 amino acids correspond with the lantibiotic, Salivaricin A, produced by *Streptococcus salivarius* AFF-22. Again this may indicate horizontal gene transfer [49, 95]. Thus, rather than debating the relative merits of broad-range one peptide and two peptide lantibiotics, it may be more appropriate to view the most active forms of each as indicators of the direction in which related peptides are likely to evolve.

Two peptide lantibiotics display all of the features that have made lantibiotics the focus of rigorous research in recent years. In addition to possessing dual mechanisms of action, these antimicrobials are active at nanomolar concentrations, bind lipid II, undergo the post-translational modifications typical of lantibiotics (in addition to some other less typical examples). However, it is perhaps most significant that these peptides have a broad range of activity and that, in the case of lacticin 3147, the development of resistance is rare. This is especially significant as the emergence of multi-resistant pathogens has heralded an outcry from medical practitioners as newer generations of conventional antibiotics are ever more quickly becoming ineffective when treating these infections. The data to date indicates that two peptide lantibiotics could be utilized to eliminate or, at the very least, help in the control of these multi-resistant microorganisms. It may be that even if two peptide lantibiotics alone are not the answer to medicine's current dilemma, their use in combination with other lantibiotics/cell membrane-acting agents could be employed as an even more effective therapy. The fact that rational and random mutagenesis of these peptides, both *in vivo* and *in vitro*, is possible suggests that a selection of two peptide lantibiotics, that is much larger than the existing natural pool, will ultimately become available in the laboratory in the future.

ACKNOWLEDGEMENTS

Appreciation is expressed to the Irish Government under the National Development Plan (2000-2006), and Science Foundation Ireland for supporting research in our laboratories.

REFERENCES

[1] Cotter, P.D.; O'Connor, P.M.; Draper, L.A.; Lawton, E.M.; Deegan, L.H.; Hill, C.; Ross, R.P. *Proc. Natl. Acad. Sci. USA*, **2005**, *102*, 18584.
[2] Poey, M.E.; Azpiroz, M.F.; Lavina, M. *Antimicrob. Agents Chemother*., **2006**, *50*, 1411.
[3] Duport, C.; Baysse, C.; Michel-Briand, Y. *J. Biol. Chem*., **1995**, *270*, 8920.
[4] Stahl, C.H.; Callaway, T.R.; Lincoln, L.M.; Lonergan, S.M.; Genovese, K.J. *Antimicrob. Agents Chemother*., **2004**, *48*, 3119.
[5] Cotter, P.D.; Hill, C.; Ross, R.P. *Nat. Rev. Microbiol*., **2005**, *3*, 777.
[6] Cotter, P.D.; O'Connor, P.M.; Draper, L.A.; Lawton, E.M.; Deegan, L.H.; Hill, C.; Ross, R.P. *Proc. Natl. Acad. Sci. USA*, **2005**, *102*, 18584.
[7] Banerjee, S.; Hansen, J.N. *J. Biol. Chem*., **1988**, *263*, 9508.
[8] Knox, J.R.; Keck, P.C. *Biochem. Biophys. Res. Commun*., **1973**, *53*, 567.
[9] Jung, G. *Angewandte Chemie (Int. Ed. Engl.*), **1991**, *30*, 1051.
[10] Jack, R.W.; Jung, G. *Curr. Opin. Chem. Biol*., **2000**, *4*, 310.
[11] Pag, U.; Sahl, H.G. *Curr. Pharm. Des*., **2002**, *8*, 815.
[12] Allgaier, H.; Jung, G.; Werner, R.G.; Schneider, U.; Zahner, H. *Eur. J. Biochem*., **1986**, *160*, 9.
[13] Gross, E.; Morell, J.L. *J. Am. Chem. Soc*., **1971**, *93*, 4634.
[14] Kellner, R.; Jung, G.; Horner, T.; Zahner, H.; Schnell, N.; Entian, K.D.; Gotz, F. *Eur. J. Biochem*., **1988**, *177*, 53.
[15] Meyer, H.E.; Heber, M.; Eisermann, B.; Korte, H.; Metzger, J.W.; Jung, G. *Anal. Biochem*., **1994**, *223*, 185.
[16] Martin, N.I.; Sprules, T.; Carpenter, M.R.; Cotter, P.D.; Hill, C.; Ross, R.P.; Vederas, J.C. *Biochemistry*, **2004**, *43*, 3049.
[17] Paik, S.H.; Chakicherla, A.; Hansen, J.N. *J. Biol. Chem*., **1998**, *273*, 23134.
[18] Sahl, H.G.; Jack, R.W.; Bierbaum, G. *Eur. J. Biochem*., **1995**, *230*, 827.
[19] Kupke, T.; Kempter, C.; Jung, G.; Gotz, F. *J. Biol. Chem*., **1995**, *270*, 11282.
[20] Kupke, T.; Stevanovic, S.; Sahl, H.G.; Gotz, F. *J. Bacteriol*., **1992**, *174*, 5354.
[21] Kupke, T.; Kempter, C.; Gnau, V.; Jung, G.; Gotz, F. *J. Biol. Chem*., **1994**, *269*, 5653.
[22] Majer, F.; Schmid, D.G.; Altena, K.; Bierbaum, G.; Kupke, T. *J. Bacteriol*., **2002**, *184*, 1234.
[23] Cotter, P.D.; Hill, C.; Ross, R.P. *Curr. Protein Pept. Sci*., **2005**, *6*, 61.
[24] McAuliffe, O.; Ross, R.P.; Hill, C. *FEMS Microbiol. Rev*., **2001**, *25*, 285.
[25] Widdick, D.A.; Dodd, H.M.; Barraille, P.; White, J.; Stein, T.H.; Chater, K.F.; Gasson, M.J.; Bibb, M.J. *Proc. Natl. Acad. Sci. USA*, **2003**, *100*, 4316.
[26] Ryan, M.P.; Jack, R.W.; Josten, M.; Sahl, H.G.; Jung, G.; Ross, R.P.; Hill, C. *J. Biol. Chem*., **1999**, *274*, 37544.
[27] Navaratna, M.A.; Sahl, H.G.; Tagg, J.R. *Appl. Environ. Microbiol*., **1998**, *64*, 4803.
[28] Holo, H.; Jeknic, Z.; Daeschel, M.; Stevanovic, S.; Nes, I.F. *Microbiology*, **2001**, *147*, 643.
[29] Yonezawa, H.; Kuramitsu, H.K. *Antimicrob. Agents Chemother*., **2005**, *49*, 541.
[30] Hyink, O.; Balakrishnan, M.; Tagg, J.R. *FEMS Microbiol. Lett*., **2005**, *252*, 235.
[31] McClerren, A.L.; Cooper, L.E.; Quan, C.; Thomas, P.M.; Kelleher, N.L.; Van der Donk, W.A. *Proc. Nat. Acad. Sci. USA*, **2006**, *103*, 17243.
[32] Booth, M.C.; Bogie, C.P.; Sahl, H.G.; Siezen, R.J.; Hatter, K.L.; Gilmore, M.S. *Mol. Microbiol*., **1996**, *21*, 1175.
[33] Guder, A.; Schmitter, T.; Wiedemann, I.; Sahl, H.G.; Bierbaum, G. *Appl. Environ. Microbiol*., **2002**, *68*, 106.
[34] Brotz, H.; Bierbaum, G.; Leopold, K.; Reynolds, P.E.; Sahl, H.G. *Antimicrob. Agents Chemother*., **1998**, *42*, 154.
[35] Hasper, H.E.; Kramer, N.E.; Smith, J.L.; Hillman, J.D.; Zachariah, C.; Kuipers, O.P.; de Kruijff, B.; Breukink, E. *Science*, **2006**, *313*, 1636.
[36] Wiedemann, I.; Breukink, E.; van Kraaij, C.; Kuipers, O.P.; Bierbaum, G.; de Kruijff, B.; Sahl, H.G. *J. Biol. Chem*., **2001**, *276*, 1772.

[37] Breukink, E.; Wiedemann, I.; van Kraaij, C.; Kuipers, O.P.; Sahl, H.; de Kruijff, B. *Science*, **1999**, *286*, 2361.
[38] Hsu, S.T.; Breukink, E.; Tischenko, E.; Lutters, M.A.; de Kruijff, B.; Kaptein, R.; Bonvin, A.M.; van Nuland, N.A. *Nat. Struct. Mol. Biol*., **2004**, *11*, 963.
[39] Yuan, J.; Zhang, Z.Z.; Chen, X.Z.; Yang, W.; Huan, L.D. *Appl. Microbiol. Biotechnol*., **2004**, *64*, 806.
[40] Brumfitt, W.; Salton, M.R.; Hamilton-Miller, J.M. *J. Antimicrob. Chemother*., **2002**, *50*, 731.
[41] Bonelli, R.R.; Schneider, T.; Sahl, H.G.; Wiedemann, I. *Antimicrob. Agents Chemother*., **2006**, *50*, 1449.
[42] Brotz, H.; Bierbaum, G.; Markus, A.; Molitor, E.; Sahl, H.G. *Antimicrob. Agents Chemother*., **1995**, *39*, 714.
[43] Kessler, H.; Seip, S.; Wein, T. In Nisin and novel lantibiotics; G. Jung, G.; Sahl, H.G. Eds.; ESCOM Science publishers B.V: Leiden, **1991**; pp. 141.
[44] Kido, Y.; Hamakado, T.; Yoshida, T.; Anno, M.; Motoki, Y.; Wakamiya, T.; Shiba, T. *J. Antibiot. (Tokyo)*, **1983**, *36*, 1295.
[45] Marki, F.; Hanni, E.; Fredenhagen, A.; van Oostrum, *J. Biochem. Pharmacol*., **1991**, *42*, 2027.
[46] Machaidze, G.; Seelig, J. *Biochemistry*, **2003**, *42*, 12570.
[47] Makino, A.; Baba, T.; Fujimoto, K.; Iwamoto, K.; Yano, Y.; Terada, N.; Ohno, S.; Sato, S.B.; Ohta, A.; Umeda, M.; Matsuzaki, K.; Kobayashi, T. *J. Biol. Chem*., **2003**, *278*, 3204.
[48] Delves-Broughton, J.; Blackburn, P.; Evans, R.J.; Hugenholtz, J. *Antonie Van Leeuwenhoek*, **1996**, *69*, 193.
[49] Georgalaki, M.D.; Van Den Berghe, E.; Kritikos, D.; Devreese, B.; Van Beeumen, J.; Kalantzopoulos, G.; De Vuyst, L.; Tsakalidou, E. *Appl. Environ. Microbiol*., **2002**, *68*, 5891.
[50] Severina, E.; Severin, A.; Tomasz, A. *J. Antimicrob. Chemother*., **1998**, *41*, 341.
[51] Kerr, K.G.; Copley, R.M.; Wilcoy, M.H. *Lancet*, **1997**, *349*, 1026.
[52] Blackburn, P.; Goldstein, B.P. Applied Microbiology, Inc., International Patent Application, **1995**, WO 97/10801.
[53] Broadbent, J.R.; Chou, Y.C.; Gillies, K.; Kondo, J.K. *J. Dairy Sci*., **1989**, *72*, 3342.
[54] Aranha, C.; Gupta, S.; Reddy, K.V. *Contraception*, **2004**, *69*, 333.
[55] Reddy, K.V.; Aranha, C.; Gupta, S.M.; Yedery, R.D. *Reproduction*, **2004**, *128*, 117.
[56] Kruszewska, D.; Sahl, H.G.; Bierbaum, G.; Pag, U.; Hynes, S.O.; Ljungh, A. *J. Antimicrob. Chemother*., **2004**, *54*, 648.
[57] Nascimento, J.S.; Ceotto, H.; Nascimento, S.B.; Giambiagi-Demarval, M.; Santos, K.R.; Bastos, M.C. *Lett. Appl. Microbiol*., **2006**, *42*, 215.
[58] Fontana, M.B.; de Bastos Mdo, C.; Brandelli, A. *Curr. Microbiol*., **2006**, *52*, 350.
[59] Smith, L.; Novak, J.; Rocca, J.; McClung, S.; Hillman, J.D.; Edison, A.S. *Eur. J. Biochem*., **2000**, *267*, 6810.
[60] Wescombe, P.A.; Upton, M.; Dierksen, K.P.; Ragland, N.L.; Sivabalan, S.; Wirawan, R.E.; Inglis, M.A.; Moore, C.J.; Walker, G.V.; Chilcott, C.N.; Jenkinson, H.F.; Tagg, J.R. *Appl. Environ. Microbiol*., **2006**, *72*, 1459.
[61] Tagg, J.R. *NZ Dent. J*., **1991**, *87*, 14.
[62] Tagg, J.R. *Indian J. Med. Res*., **2004**, *119*(Suppl.), 13.
[63] Dierksen, K.P.; Moore, C.J.; Inglis, M.; Wescombe, P.A.; Tagg, J.R. *FEMS Microbiol. Ecol*., **2007**, *59*, 584.
[64] Bowe, W.P.; Filip, J.C.; DiRienzo, J.M.; Volgina, A.; Margolis, D.J. *J. Drugs Dermatol*., **2006**, *5*, 868.
[65] Morgan, S.M.; O'Connor P, M.; Cotter, P.D.; Ross, R.P.; Hill, C. *Antimicrob. Agents Chemother*., **2005**, *49*, 2606.
[66] Kawai, Y.; Ishii, Y.; Arakawa, K.; Uemura, K.; Saitoh, B.; Nishimura, J.; Kitazawa, H.; Yamazaki, Y.; Tateno, Y.; Itoh, T.; Saito, T. *Appl. Environ. Microbiol*., **2004**, *70*, 2906.
[67] McAuliffe, O.; Hill, C.; Ross, R.P. *Microbiology*, **2000**, *146*, 2147.
[68] Cotter, P.D.; Deegan, L.H.; Lawton, E.M.; Draper, L.A.; O'Connor, P.; Hill, C.; Ross, R.P. *Mol. Microbiol*., **2006**, *62*, 735.
[69] Lawton, E.M.; Cotter, P.D.; Hill, C.; Ross, R.P. *FEMS Microbiol. Lett*., **2007**, *267*, 64.
[70] Dajani, A.S.; Gray, E.D.; Wannamaker, L.W. *J. Exp. Med*., **1970**, *131*, 1004.
[71] Navaratna, M.A.; Sahl, H.G.; Tagg, J.R. *Infect. Immun*., **1999**, *67*, 4268.
[72] Warren, R.; Rogolsky, M.; Wiley, B.B.; Glasgow, L.A. *J. Bacteriol*., **1975**, *122*, 99.
[73] Yamaguchi, T.; Hayashi, T.; Takami, H.; Ohnishi, M.; Murata, T.; Nakayama, K.; Asakawa, K.; Ohara, M.; Komatsuzawa, H.; Sugai, M. *Infect. Immun*., **2001**, *69*, 7760.
[74] O'Connor, E.B.; O'Riordan, B.; Morgan, S.M.; Whelton, H.; O'Mullane, D.M.; Ross, R.P.; Hill, C. *J. Appl. Microbiol*., **2006**, *100*, 1251.
[75] Petersen, F.C.; Fimland, G.; Schele, A.A. *Mol. Microbiol*., **2006**, *61*, 1322
[76] Coburn, P.S.; Pillar, C.M.; Jett, B.D.; Haas, W.; Gilmore, M.S. *Science*, **2004**, *306*, 2270.
[77] Coburn, P.S.; Gilmore, M.S. *Cell Microbiol*., **2003**, *5*, 661.
[78] Haas, W.; Shepard, B.D.; Gilmore, M.S. *Nature*, **2002**, *415*, 84.
[79] Gilmore, M.S.; Segarra, R.A.; Booth, M.C.; Bogie, C.P.; Hall, L.R.; Clewell, D.B. *J. Bacteriol*., **1994**, *176*, 7335.
[80] Field, D.; Collins, B.; Cotter, P.D.; Hill, C.; Ross, R.P. *J. Mol. Mic. Biotechnol*., **2007**, In print.
[81] Chen, P.; Qi, F.X.; Novak, J.; Krull, R.E.; Caufield, P.W. *FEMS Microbiol. Lett*., **2001**, *195*, 139.
[82] Neis, S.; Bierbaum, G.; Josten, M.; Pag, U.; Kempter, C.; Jung, G.; Sahl, H.G. *FEMS Microbiol. Lett*., **1997**, *149*, 249.
[83] van der Meer, J.R.; Rollema, H.S.; Siezen, R.J.; Beerthuyzen, M.M.; Kuipers, O.P.; de Vos, W.M. *J. Biol. Chem*., **1994**, *269*, 3555.
[84] Galvin, M.; Hill, C.; Ross, R.P. *Lett. Appl. Microbiol*., **1999**, *28*, 355.
[85] Zaoutis, T.; Attia, M.; Gross, R.; Klein, J. *Clin. Microbiol. Infect*., **2004**, *10*, 37.
[86] Torres, A.M.; Menz, I.; Alewood, P.F.; Bansal, P.; Lahnstein, J.; Gallagher, C.H.; Kuchel, P.W. *FEBS Lett*., **2002**, *524*, 172.
[87] Kumar, A.; Sandoe, J.; Kumar, N. *J. Med. Microbiol*., **2005**, *54*, 1103.
[88] Twomey, D.; Ross, R.P.; Ryan, M.; Meaney, B.; Hill, C. *Antonie Van Leeuwenhoek*, **2002**, *82*, 165.
[89] Cotter, P.D.; Guinane, C.M.; Hill, C. *Antimicrob. Agents Chemother*., **2002**, *46*, 2784.
[90] Ladhani, S.; Joannou, C.L.; Lochrie, D.P.; Evans, R.W.; Poston, S.M. *Clin. Microbiol. Rev*., **1999**, *12*, 224.
[91] Ladhani, S.; Poston, S.M.; Joannou, C.L.; Evans, R.W. *Acta Paediatr*., **1999**, 88, 776.
[92] Plano, L.R. *J. Invest. Dermatol*., **2004**, *122*, 1070.
[93] Chow, J.W.; Thal, L.A.; Perri, M.B.; Vazquez, J.A.; Donabedian, S.M.; Clewell, D.B.; Zervos, M.J. *Antimicrob. Agents Chemother*., **1993**, *37*, 2474.
[94] Dabard, J.; Bridonneau, C.; Phillipe, C.; Anglade, P.; Molle, D.; Nardi, M.; Ladire, M.; Girardin, H.; Marcille, F.; Gomez, A.; Fons, M. *Appl. Environ. Microbiol*., **2001**, *67*, 4111.
[95] Van den Berghe, E.; Skourtas, G.; Tsakalidou, E.; De Vuyst, L. *Int. J. Food Microbiol*., **2006**, *107*, 138.

Received: 04 January, 2007 **Revised: 11 May, 2007** **Accepted: 15 May, 2007**