Expert Opinion

Discovery and development of lantibiotics; antimicrobial agents that have significant potential for medical application

Mohammed M Al-Mahrous & Mathew Upton†
University of Manchester, School of Translational Medicine, Department of Medical Microbiology, Manchester, UK

Introduction: Antimicrobial drug resistance is driving the need for novel therapeutics. Amongst the most promising antibacterial agents that are being investigated as replacements for current therapeutic antibiotics are antibacterial peptides, such as the lanthionine-containing peptide antibiotics (lantibiotics).

Areas covered: This review focuses on the current methods used for discovery of potentially exploitable lantibiotics for medical applications and discusses relevant recent innovations that will have a positive impact on the discovery of useful lantibiotics.

Expert opinion: Recent technological advances in a number of fields mean that increased research into the identification and characterisation of new lantibiotics is feasible. We need to increase our understanding of the various mechanisms of antibacterial action exhibited by lantibiotics and apply this knowledge to peptide engineering or novel practical applications. The advent of next-generation sequencing approaches now negate the need for extensive reverse genetics and employment of bioinformatics approaches is greatly assisting the identification of potentially useful inhibitors in the genomes of a range of clinically significant bacteria. These advances in genetic analysis and engineering will facilitate increased exploitation of lantibiotics in medical therapy.

Keywords: bioengineering, bioinformatics, biosynthetic pathways, genetic engineering, genome sequence, lantibiotics, mechanism of action

Expert Opin. Drug Discov. [Early Online]

1. Introduction

1.1 The significance of naturally produced cationic peptide antibiotics is reflected in their evolutionary origin as inhibitory substances that microbes use against each other

The knowledge that naturally produced cationic peptide substances evolved as inhibitory substances that microbes use against each other demonstrates their ecological relevance. In the past, these agents have been very useful as a supply of clinically important antibacterial drugs, as in the case of glycopeptides. One of the most significant examples in this regard is vancomycin, which was isolated from the fermentation broth of the actinomycete, *Amycolatopsis orientalis* [1].

There are also other classes of antibacterial substances that are naturally produced by Gram-positive bacteria, which have not yet been fully appraised for application in medical practice. The class I bacteriocins, the lantibiotics (**Table 1**), are among the most interesting of these developmental agents [2] and have recently attracted the interest of many groups [3].

informa
healthcare

10.1517/17460441.2011.545387 © 2011 Informa UK, Ltd. ISSN 1746-0441

Article highlights.

- Conventional antibiotics are becoming increasingly less useful in the fight against drug resistant bacteria and novel therapies are urgently needed.
- Lantibiotics have been trialled in some veterinary settings, but their full potential in clinical use in humans has not been realised.
- Conventionally, screening programmes and downstream identification of genetic pathways required for lantibiotic production have been time consuming and laborious.
- Recent advances in DNA sequence determination, bioprocess engineering and bioinformatics should be exploited to expedite the development of lantibiotics in the medical field.

This box summarises key points contained in the article.

1.2 The increase in antibiotic resistance requires discovery of novel therapeutics, rather than modification of existing agents

Since the discovery of penicillin > 80 years ago by Alexander Fleming, antibacterial drugs have had an enormous impact on the treatment of diseases caused by bacteria [4-6]. The inappropriate and widespread use of antibacterial drugs has presented a strong evolutionary pressure for the development and spread of resistant and harmful bacteria that either have an inherent resistance to a certain antibiotic or have the ability to gain such resistance [7]. In spite of the introduction of a number of new antibacterial substances in several distinct drug classes, resistance persists [5]. Accordingly, at present there is no clinically used antibacterial drug to which resistance has not arisen, and so there is a serious demand to discover and develop novel pipelines of antibacterial substances that are active against pathogenic bacteria, including resistant strains that inhabit healthcare settings, such as the staphylococci [5,7].

However, the clear and urgent need for novel antibacterial substances has not driven industry to move its focus to that of novel therapeutic discovery [4,6,7], even though infectious diseases are still amongst the leading causes of mortality in the world [8]. This fall in antibacterial agent discovery, together with the growing threat resulting from diseases caused by resistant bacterial pathogens, represents a very real public health risk [4,6-8]. Therefore, industry must act in response to these clinical challenges by bringing forward a flow of novel substances with antibacterial activity against pathogenic bacteria [5] and a clear and urgent need for finding ways to advance the level and quality of industrial research in this field is evident [4,6-8].

In this review, the focus is on the investigation of one potentially useful class of novel antibiotics, the lantibiotics. Lantibiotics are cationic peptides, members of the bacteriocins and have a number of promising features. Over 50 lantibiotics that have been discovered to date display antimicrobial inhibitory activity at nanomolar levels against a range of hospital-acquired pathogens such as methicillin resistant *Staphylococcus aureus* and vancomycin resistant *Enterococcus faecalis* [9,10]. In addition, even after > 40 years of widespread use as a food preservative, food-borne pathogens have not developed any considerable levels of resistance to the most prominent representative of the large number of lantibiotics, nisin [11]. The lack of development of resistance in susceptible bacteria, the potent activity and, more recently, the scope for modification of physicochemical properties and activity thorough bioengineering approaches [12] are the features of lantibiotics that place them above conventional agents as potential immensely promising future therapeutics.

1.3 Lantibiotics: bacteriocins that represent novel therapeutic agents

The bacteriocins are produced by bacteria and are generally active against the same or closely related species [8,10,13], though there have been recent reports of lantibiotics that lack antimicrobial activity [14]. Based on Gram-staining of the producing bacteria, bacteriocins can be divided into two major groups [3,15,16].

The bacteriocins of Gram-negative bacteria were first to be studied extensively. The colicins, which are produced by members of the *Enterobacteriaceae*, are considered the prototypes [3,16]. They are large proteinaceous compounds with domains specific for certain functions, such as binding to receptor proteins in the outer membrane and translocation through the peptidoglycan layer and periplasmic space [16,17].

The bacteriocins of Gram-positive bacteria (**Tables 1** and **2**) have been classified into numerous subtypes [17,18]. The principal groups are: i) bacteriocins that contain lanthionine, the lantibiotics; ii) bacteriocins-containing cysteine ring structures instead of lanthionine and β-methyl-lanthionine; iii) bacteriocins whose mode of action is dependent on their content of a single cysteine residue; iv) bacteriocins not containing free cysteine residues and v) bacteriocins whose action is dependent on the cooperative action of more than one peptide.

While the majority of the bacteriocins are linear, unmodified peptides, which may contain disulfide bonds [3], lantibiotics (*lan*thionine-containing an*tibiotics*) (**Table 1**) are small, complex peptides with post-translational modifications. Dehydration of amino acids and non-ribosomal introduction of the thioether amino acid lanthionine (Lan) or 3-methyllanthionine leads to ring formation, giving scope for variability in structures encoded by lantibiotic genes [19].

A feature common of all of the Gram-positive bacteriocin subtypes is that they are produced ribosomally as a prepeptide consisting of two parts or pseudodomains, the leader region and the propeptide components. After the synthesis of the prepeptide (leader + propeptide), amino-acid modifications that may be necessary for the biological functionality of the peptide occur. It is this mechanism of ribosomal biosynthesis, sometimes followed by post-translational modification, which principally distinguishes the bacteriocins from other peptide

Table 1. General characteristics of some currently described lantibiotics.

Lantibiotic[#]	Producer species	Mass (Da)	Residues*	Charge[‡]	Rings[§]	Modified residues[¶]
Type A lantibiotics						
Nisin A	*Lactococcus lactis*	3353	34	+3	5	Dha, Dhb
Nisin Z	*Lactococcus lactis*	3330	34	+3	5	Dha, Dhb
Subtilin	*Bacillus subtilis*	3317	32	+2	5	Dha, Dhb
Pep5	*Staphylococcus epidermidis*	3488	34	+7	3	Dha, Ob
Epilancin K7	*Staphylococcus epidermidis*	3032	31	+5	3	Dha, Dhb, Hop
Epilancin 15X	*Staphylococcus epidermidis*	3173	31	nk	3	Hop, Dha, Dhb
Epicidin 280	*Staphylococcus epidermidis*	3133	30	+4	3	Dhb, Hop
Epidermin	*Staphylococcus epidermidis*	2164	22	+3	4	Dhb, AviCys
(Val-1, Leu-6)-epidermin	*Staphylococcus epidermidis*	2151	22	+3	4	Dhb, AviCys
Gallidermin (leu-6)-epidermin	*Salmonella gallinarium*	2164	22	+3	4	Dhb, AviCys
SA-FF22	*Streptococcus pyogenes*	2795	26	+1	3	Dhb
Lacticin 481	*Lactococcus lactis*	2901	27	0	3	Dhb
Salivaricin A	*Streptococcus salivarius*	2315	22	0	3	None
(Lys-2, Phe-7)-salivaricin A	*Streptococcus pyogenes*	2321	22	0	3	None
(Thr-4, Phe-7)-salivaricin A	*Streptococcus salivarius*	2368	22	0	3	None
Variacin	*Micrococcus varians*	2658	25	0	3	Dhb
Sublancin 168	*Bacillus subtilis*	3878	37	nk	1	Dha, 2 Dsb
Lactocin S	*Lactococcus sake*	3764	37	-1	2	Dhb, D-Ala, Ob
Mutacin B-Ny266	*Streptococcus mutans*	2270	21	nk	3	Dha, Dhb
Mutacin 1140	*Streptococcus mutans*	2263	21	nk	3	Dha, Dhb
Mutacin III	*Streptococcus mutans*	2266	22	nk	3	Dha, Dhb
Mutacin II	*Streptococcus mutans*	3245	27	nk	3	Dhb
Carnocin Ui49	*Carnobacterium piscicola*	4635	nk	nk	nk	nk
Cypemycin	*Streptomyces* species	2094	22	0	1	*allo*-Ile,AviCys
Butyrivibriocin OR79A	*Butyrivibrio fibrisolvens*	nk	25	nk	3	Dhb
Staphylococcin 1580	*Staphylococcus epidermidis*	2165	nk	nk	nk	nk
Staphylococcin T	*Staphylococcus cohnii*	2166	22	nk	nk	nk
Nukacin ISK-1	*Staphylococcus warneri*	2960	27	nk	3	Dhb
Nukacin KQU-131	*Staphylococcus hominis*	3003	27	nk	3	Dhb
Type B lantibiotics						
Cinnamycin	*Streptoverticillium cinnamoneus*	2042	19	0	4	Asp-OH, LysAla
Duramycin	*Streptoverticillium cinnamoneus*	2014	19	0	4	Asp-OH, LysAla
Duramycin B	*Streptoverticillium* species	1951	19	0	4	Asp-OH, LysAla
Duramycin C	*Streptoverticillium cinnamoneus*	2008	19	-1	4	Asp-OH, LysAla
Ancovenin	*Streptomyces* species	1959	19	0	3	Dha
Mersacidin	*Bacillus* species	1825	20	-1	4	Dha, MeAviCys
Actagardine	*Actinoplanes* species	1890	19	0	4	LanO
Two-component lantibiotics						
Cytolysin A1	*Enterococcus faecalis*	4164	38	0	nk	Dha, Dhb
Cytolysin A2		2631	21	0	nk	Dha, Dhb
Staphylococcin C55α	*Staphylococcus aureus*	3339	29	nk	nk	Dha, Dhb
Staphylococcin C55β		2993	32	nk	nk	Dhb
Plantaricin Wα	*Lactococcus plantarum*	3223	29	nk	3	
Plantaricin Wβ		3099	32	nk	3	Dha, Dhb
Lacticin 3147 A1	*Lactococcus lactis*	3322	30	nk	nk	Dha, Dhb, D-Ala
Lacticin 3147 A2		2847	29	nk	3	Dha, Dhb, D-Ala, Ob
Haloduracin A1	*Bacillus halodurans*	2332	28	nk	nk	nk
Haloduracin A2		3046	30	nk	nk	nk

*Includes modified residues and N-terminal modifications. Each lanthionine (Lan), β-methyl-lanthionine (MeLan), aminovinyl-cysteine (AviCys) and 3-methyl AviCys (MeAviCys) is counted as two residues.

[‡]At pH 7.0 (includes free N and/or C terminus).

[§]Rings resulting from covalent linkage of various residues, including Lan, MeLan, AviCys, MeAviCys and lysinoalanine (LysAla).

[¶]Modified residues: Dha, α/β-didehydroalanine; Dhb, α/β-didehydrobutyrine; D-Ala, D-alanine; allo-Ile, allo-isoleucine; Asp-OH, hydroxyaspartic acid; LanO, lanthionine oxide; 2-hydroxypropionyl (Hop); 2-oxobutyryl (Ob); 2 disulfide bridges (Dsb).

[#]Lantibiotics are divided into two groups based on the proposal of Jung and Sahl [46] and a third group including the two-component lantibiotics.

nk: Not known.

Table 2. The currently known staphylococcal bacteriocins.

Bacteriocins*	Species	Mass (Da)	Residues
Class I, type A lantibiotics			
Pep5‡	*Staphylococcus epidermidis*	3488	34
Epilancin K7‡,§	*Staphylococcus epidermidis*	3032	31
Epicidin 280‡	*Staphylococcus epidermidis*	3133	30
Epilancin 15X§	*Staphylococcus epidermidis*	3173	31
Epidermin§	*Staphylococcus epidermidis*	2164	22
(Val-1, Leu-6)-epidermin§,**	*Staphylococcus epidermidis*	2151	22
Gallidermin (leu-6 epidermin)§,**	*Salmonella gallinarium*	2164	22
Staphylococcin 1580§,**	*Staphylococcus epidermidis*	2165	22
Staphylococcin T‡‡	*Staphylococcus cohnii*	2166	22
Nukacin ISK-1	*Staphylococcus warneri*	2960	27
Nukacin KQU-131	*Staphylococcus hominis*	3003	27
BSa1	*Staphylococcus aureus*	2281	23
BSa2	*Staphylococcus aureus*	2091	21
Class I, two-component lantibiotics			
Staphylococcin C55α¶	*Staphylococcus aureus*	3339	nk
Staphylococcin C55β¶		2993	nk
Class IId, one-peptide non-pediocin-like linear bacteriocins (without a leader sequence)			
Aureocin A53	*Staphylococcus aureus*	6012	51
Aureocin A70#	*Staphylococcus aureus*	nk	30 and 31
Class IV, complex bacteriocins that contain carbohydrate and/or lipid moieties			
Aureocin 215FN	*Staphylococcus aureus*	nk	nk
Aureocin MB92	*Staphylococcus aureus*	nk	nk
Aureocin 146L	*Staphylococcus aureus*	nk	nk
Unclassified bacteriocins			
$BacR_1$¶	*Staphylococcus aureus*	3338	nk
Bac1829	*Staphylococcus aureus*	6418	nk
IYS2	*Staphylococcus aureus*	5000	nk
Staphylococcin 263	*Staphylococcus aureus*	nk	nk
Staphylococcin 414	*Staphylococcus aureus*	nk	nk
Staphylococcin 462	*Staphylococcus aureus*	nk	nk
Staphylococcin 412	*Staphylococcus aureus*	200,000	nk
Staphylococcin A	*Staphylococcus aureus*	nk	nk
Staphylococcin D91	*Staphylococcus aureus*	76,000§§	nk
Bac201	*Staphylococcus aureus*	41,000	nk

*Bacteriocins of Gram-positive bacteria can also be divided into five groups based on previous proposals [17,18]. Class I (lantibiotics), containing small post-translationally-modified residues. A distinction is made between type A lantibiotics, positively-charged flexible elongated peptides and type B lantibiotics, which are rigid globular molecules with either no or a net negative charge. Class II (small unmodified-membrane active peptides) is divided into four subclasses: class IIa (pediocin-like bacteriocins), with strong inhibitory effect on *Listeria monocytogenes* and containing a conserved YGNG sequence motif in the N-terminal half of the mature protein; class IIb (two peptide bacteriocins), which require complementary action of two peptides for full activity; class IIc (*Sec*-dependant) are exported via the *Sec* machinery rather than via dedicated ABC transporters and class IId that does not contain a leader sequence and are produced with further processing. Class III is composed of large heat-labile bacteriocins. Class IV covers complex proteins that require carbohydrate or lipid moieties for bacteriocin activity. Class V is circular, head-to-tail ligated bacteriocins.

‡Closely-similar lantibiotics in this table are: epilancin 280 and Pep5 (75%); epilancin K7 and epilancin 15X.

§Almost identical lantibiotics in this table are: gallidermin and epidermin; (Val-1, Leu-6)-epidermin and epidermin; staphylococcin 1580 and epidermin; staphylococcin T and gallidermin.

¶Produced by phage group II *Staphylococcus aureus*, and associated with production of exfoliative toxin B (ET-B), but not A (ET-A).

#It is not clear if aureocin A70 is a novel four-peptide bacteriocin that each contains 30 and 31-residue, or simply four separate one-peptide bacteriocins with similar sequences. They are small, strongly cationic (pI values of 9.85 – 10.04) and highly hydrophobic peptides with theoretical mass of 2924 Da (31 residues), 2797 Da (30 residues), 2954 Da (31 residues) and 3086 Da (31 residues), respectively.

**Epidermin variant.

‡‡Gallidermin variant.

§§Most probable.

nk: Not known; pI: Isoelectric point.

antibiotics, that is, those produced in bacterial cells by multi-enzyme complexes, for which no gene encoding a ribosomally synthesised peptide exists [2].

1.4 A generic nomenclature for the functional genetic determinants of all lantibiotics is suggested

The genetic determinants encoding enzymes involved in lantibiotic processing have now been described for various members of types A and B (**Table 1**). A generic nomenclature for the functional genetic determinants of all lantibiotics has been recommended [20]. These include structural genes encoding the precursor peptide (LanA), modification enzymes (LanB, C, M and D), proteases responsible for cleavage of the leader peptide (LanP), ABC transport proteins involved in peptide translocation (LanT), regulatory proteins (LanR, K, Q) and immunity mechanisms (LanI, F, E, G), in addition to other genes with no homologues in the databases [2,16,21,22], which may be involved in immunity (**Figure 1**).

The role of some of these enzymes is now being studied in more detail and it has been shown that lacticin 481 is modified by LctM, which can successfully process different LctA mutants [23]. As such, LctM exhibits a relaxed substrate specificity allowing modification of lantibiotic variants [24].

More detailed studies examining biosynthetic intermediates have shown that LctM, and similarly HalM2, are bi-functional and catalyse dehydration reactions and Michael-type additions of cysteine residues to the dehydro-serines and -theronines [25].

It has been reported that other rare or special residues occur for which the mechanism of biosynthesis is not known in all cases [26]. Cotter *et al.* have also reported that dehydrated serine residues can be converted to D-alanine by a novel enzyme designated LanJ [9].

Two-component, *lanK* and *lanR* genes have been identified in connection with a number of lantibiotics including nisin [27], subtilin [28], streptococcin A-FF22 [29], salivaricin A [30] and mersacidin [31]. LanK and LanR are representatives of two-component signal transduction systems [27]; the LanK proteins being histidine protein kinases and the LanR proteins are response regulators.

EpiQ is the regulator of epidermin biosynthesis and has some similarity to response regulators at its C terminus, but lacks the conserved phosphoryl-accepting aspartic acid residue [32].

The existence of specific immunity proteins, such as LanI, F, E and G, has been shown to be closely linked to bacteriocin production [21], although the exact mechanisms involved in immunity remain largely unknown [33].

The genes *lanF*/*E*/*G* encode a second ABC transporter, where LanF is the ATP-binding domain, and LanE and LanG are the membrane-spanning subunits. So far, this type of transporter has been identified in the gene clusters of nisin [34], epidermin [35], lacticin 481 [36] and mersacidin [31]. This mechanism of immunity was proposed to involve either the active extrusion of the lantibiotic from the membrane or uptake and subsequent intracellular degradation of the lantibiotic [35]. The first mechanism would be the most likely, and in fact cells incubated with gallidermin were shown to have a fourfold higher extracellular gallidermin concentrations in EpiFEG-producing strains compared to control strains [37].

Varying degrees of sequence conservation are observed within the functional genes. This has been exploited to good effect in a number of recent studies that have utilised degenerate PCR primers for amplification of genetic loci encoding putative bacteriocins or lantibiotics [38] and data mining approaches targeting specific sequence conservation [39-41]. These approaches have facilitated efficient access to the genetic machinery underlying production of several interesting inhibitors and similar methods may be among those seen more frequently in genome mining programmes.

Lantibiotic genetic determinants may be chromosomally encoded, as is the case for subtilin [42] and SA-FF22 [29], but in most cases they are located on large plasmids or conjugative transposons. The nisin genes, for example, are found on a number of large (~ 70 kb) conjugative transposons such as Tn*5301* in *Lactococcus lactis* NCFB894 [43] and Tn*5276* in *L. lactis* NIZO R5 [44]. These transposons also harbour genes for sucrose utilisation and integrate into the chromosome following conjugal transfer [17].

The genetic structure and sequence conservation in lantibiotic loci can also be exploited by software tools searching for relevant genes/loci. Recently, de Jong and colleagues reported that Prodigal open reading frame (ORF) prediction improves bacteriocin discovery [45]. The performance of two analysis tools available data on the National Center for Biotechnology Information (NCBI) website, namely Glimmer and Prodigal, was assessed using the genomes of four Gram-positive bacteria (*Bacillus subtilis* 168, *Lactobacillus plantarum* WCFS1, *Streptococcus pneumoniae* TIGR4 and *E. faecalis* V583). These genomes were considered to have been well annotated using the BAGEL2 web server (http://bagel2.molgenrug.nl.), which is used as an automated prediction and classifying tool based on mining bacteriocin genomic data [45]. The investigators claimed that use of the BAGEL2 web server revealed that the current annotation systems used by Joint Genome Institute, NCBI and The Institute for Genomic Research databases cannot be used to discover all putative bacteriocin genes in prokaryotic genomes. In addition, they reported that BAGEL2 adds value to bacterial genome annotation pipelines, as it quickly and precisely identifies small ORFs that code for bacteriocins [45].

Examples of lantibiotics are listed in **Table 1**. They are grouped into type A and type B peptides according to a proposal by Jung and Sahl in 1991 [46], based on their structural and functional features. Type A lantibiotics (**Table 1**) are generally elongated cationic peptides up to 34 residues in length. Many show close similarity in the arrangement of their bridge structures. These peptides act primarily by disrupting the membrane integrity of target organisms, allowing the efflux

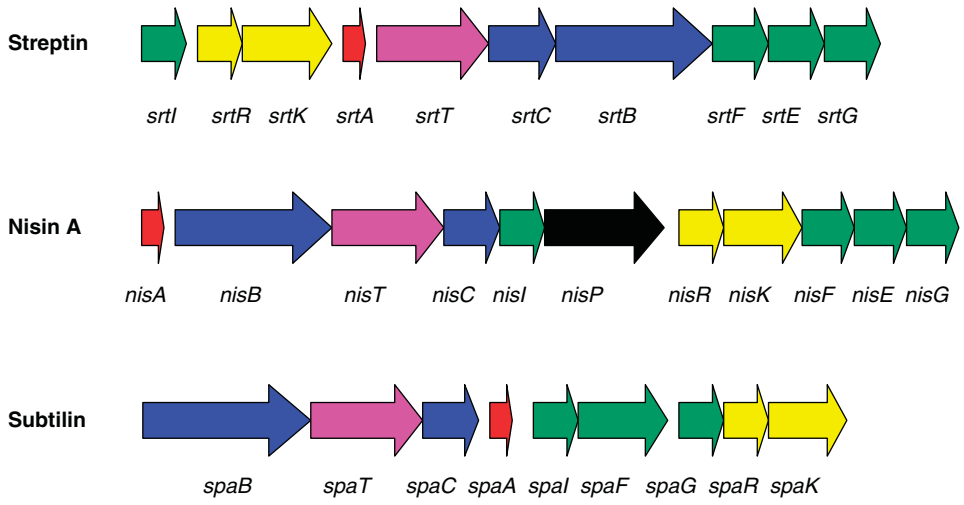

Figure 1. Comparison of the nisin A, streptin and subtilin gene clusters. Gene designations are as recorded by de Vos *et al.* [77].

of essential intracellular components, usually disrupting the proton motive force or proton gradient, limiting the production of ATP [33]. Type B lantibiotics (**Table 1**) are generally more globular structures, carry a net negative or neutral charge and comprise up to 19 residues. These, in contrast to the type A lantibiotics, act principally through disruption of enzyme function, such as inhibition of cell wall synthesis [47]. **Table 2** summarises the known staphylococcal bacteriocins. These have been the focus of recent interest in a number of groups, given the problems of drug resistance in this genus of bacteria.

Further subdivisions have been proposed and, based on the different structures of type A molecules, three subgroups have been suggested: A (I) elongated and flexible lantibiotics (e.g., nisin); A (II) lantibiotics with linear N termini and globular C termini (e.g., lacticin 481) and A (III) two-component bacteriocins (e.g., lactocin S) [15,48].

The discovery of lantibiotics with two synergistically acting and structurally different peptides [49-51] and those that interfere with enzymatic reactions (such as peptidoglycan synthesis) have bought into question some of the above classifications [10,47]. Consequently, Willey and van der Donk [10] proposed a new classification based on biosynthetic pathways, where all previously discovered lantibiotics fall into one of three classes: i) class I lantibiotics are modified by LanB and LanC enzymes (e.g., NisB and NisC for nisin) [52], exported by an ABC transporter LanT, when the leader sequence is concomitantly cleaved by a specific LanP enzyme; ii) class II lantibiotics (e.g., lacticin 481, lactocin S, SA-FF22) where the respective roles of LanB/C and LanT are carried out by LanM and LanT(P), which has an N-terminal protease activity [53] and iii) class III lantibiotics are those that lack antibiotic activity (e.g., SapT from *Streptomyces tendae* [14]).

Recently, an additional class of lanthionine synthetases (LanL) has been described in *Streptomyces venezuelae* [54]. This enzyme contains three catalytic domains that phosphorylate Ser/Thr residues leading to conversion to dehydroamino acids and subsequent removal of the phosphate by a phospho-(Ser/Thr) lyase domain. LanL enzymes have probably developed through the evolution of stand-alone protein phospho-(Ser/Thr) lyases, Ser/Thr kinases and enzymes catalyzing thiol alkylation [54].

The reported lantibiotic synthetases (LanB/C, L and M) do not share significant homology with any other known protein families suggesting an isolated evolutionary origin.

Lanthionine-containing peptides are also produced as secondary metabolites from the marine cyanobacterium *Prochlorococcus*, probably the most abundant photosynthetic organism on the Earth. Most striking in this context is the discovery that in some of these organisms, a single enzyme can modify the structure of 29 different ribosomally synthesised linear peptides. The modifications include introduction of diverse ring structures. Such promiscuity allows an organism with a small genome to maximise efficiency [55].

Similarly, nisin has five rings of different sizes. NisC catalyses the five different cyclisation reactions required to develop the rings, indicating the flexibility of NisC, which is, however, stringent enough to shape each ring in the required conformation and order [56].

2. Understanding native lantibiotic genetics and biosynthetic pathways will facilitate increased discovery and *in vitro* production

2.1 Lantibiotic loci show conservation and synteny

Examination of the organisation of the gene clusters for nisin A, streptin and subtilin (**Figure 1**) reveals significant differences in the positioning of the individual genes within the streptin locus, when compared with the nisin A and subtilin loci [57,58]. The GC-content of the streptin locus is 29.8%,

whereas that of the entire *Streptococcus pyogenes* genome (the producing organism) is 38.5% [57] suggesting an origin outside *S. pyogenes*. Similarly, the nisin Z locus has been found to have a GC-content of 32%, which is less than that observed for the lactococcal chromosome in general (average GC-content of 38%) [59]. The subtilin locus has an average GC-content of 37%, which is also less than the 43% average value for the *B. subtilis* chromosome, although this is known to fluctuate significantly throughout the genome [60]. Examination of the percent homology of each putative streptin gene with the corresponding genes in the nisin and subtilin loci showed that there is relatively high identity among the streptin, nisin A and subtilin gene products involved in lantibiotic regulation and transport [58,61]. Lower relative identity was found for gene products putatively associated with lantibiotic modification and immunity. Furthermore, by contrast with the nisin locus, the streptin and subtilin loci appear to lack a specific protease gene.

Understanding the genetic organisation of lantibiotic loci enables the use of bioinformatics approaches or (degenerate) PCR-based methods for targeted identification of homologues of known inhibitors. This can allow screening bacteria of varied genera, opening the possibility of identifying inhibitors with different activity spectra.

2.2 The chemical composition of lantibiotics undergoes extensive post-translational modification to form the biologically active peptide

Aside from lanthionine and β-methyl-lanthionine, lantibiotics may also contain a number of other modified residues, such as α/β-didehydroalanine and α/β-didehydrobutyrine unusual amino-acid derivatives. For example, an unsaturated (S)-([Z]-2-aminovinyl)-D-cysteine at the C terminus forms the fourth ring in Pep5 and epidermin [62], whereas that of epilancin K7 and lactocin S have an α/β-didehydrobutyrine at the N terminus when the propeptide is cleaved from the leader region (**Figure 2**) [22]. These dehydrated residues, however, are not stable when N terminally exposed and spontaneous deamination occurs through the addition of a water molecule, resulting in the formation of 2-oxobutryl (from α/β-didehydrobutyrine) and 2-oxopropionyl (from α/β-didehydroalanine). The 2-oxobutyryl is modified to oxopropionyl in Pep5 [46], lactocin S [63] and epicidin 280 [64] while the 2-oxopropionyl is further reduced to hydroxypropionyl in epilancin K7 [20]. Lactocin S also features D-alanine at three positions where the structural gene specifies serine. Similarly, lacticin 3147 (a two-component lantibiotic) was recently shown to have serine to D-alanine conversions in both of the peptides involved in its biological action [51]. **Figure 3** demonstrates examples of the disulfide cross linkages in different lantibiotics.

These residues may contribute to the broad antimicrobial spectrum of lacticin 3147. The functions of these unusual residues are slowly being unravelled, with the ring conformations thought to be essential for the maintenance of peptide rigidity [65], insensitivity to proteolytic degradation and resistance to thermal inactivation [66]. D-amino acids are also thought to contribute significantly to the activity and stability of the molecules [67]. The function of didehydroamino acids is still largely speculative, but they are thought to contribute to antimicrobial activity by interaction with free sulfydryl groups in the cell envelopes of target organisms [68].

Studies with nisin have demonstrated the importance of the 1st (A) and 2nd (B) ring structures in the biological activity of the molecule [69]. Interestingly, replacement of α/β-didehydroalanine with α/β-didehydrobutyrine at position 5 of nisin Z (**Figure 2**) resulted in a peptide with 2- to 10-fold less activity than native nisin [65]. The replacement of α/β-didehydroalanine at position 5 with alanine did not greatly affect the pore forming activity of nisin, but did significantly reduce the inhibition of spore outgrowth when compared with native nisin. This indicates that there are at least two different mechanisms of action associated with nisin [70].

Other lantibiotics such as Pep5 [22] and subtilin [71] have also been studied with regard to the importance of their ring structures and modified residues. Disruption or replacement of the thioether rings or modified residues in Pep5 reduced the antimicrobial activity, and in the case of alterations to the ring structures, the molecules became more susceptible to proteolytic digestion [22]. In other studies, the dehydroalanine residue at position 5 of subtilin was shown to be necessary for activity against bacterial spore outgrowth [71].

Understanding the impact on activity of such changes in amino-acid composition or sequence is informing engineering studies aimed at manipulating the activity and enhancing the utility of lantibiotics.

2.3 Lantibiotics undergo extensive modification before being released from the cell

The leader peptide encoded by the LanA gene is often cleaved from the propeptide during the last step of lantibiotic biosynthesis (**Figure 2**). For example, nisin has a 57-residue prepeptide, 23 residues of which comprise the leader and 34 the propeptide. The latter undergoes modifications and, when cleaved from the leader, is released from the cell as the mature peptide [72]. Modification of the lantibiotic prepeptide only occurs in the propeptide part. Thus, even if the leader sequence contains the hydroxyl amino acids serine and threonine, they are not modified during lantibiotic processing [73]. The leader peptides of all characterised lantibiotics are also devoid of cysteine, which together with serine or threonine residues in the propeptide part of the molecule are involved in thioether bridge formation. Lantibiotic leaders contain between 23 and 30 residues and have no resemblance to *sec*-dependant transport signal sequences, as they lack the hydrophobic membrane-spanning core and the typical processing site [74].

The lantibiotic leaders have been classified into two groups (type AI and type AII) based on their size, charge and sequence characteristics. The type AI leaders are generally

	Leader					Cleavage site	Propeptide				
Lantibiotic	-5				-1		+1				+5
Streptin (1)	K	K	D	T	A		T	P	Y	V	G
Streptin (2)	T	A	T	P	Y		V	G	S	R	Y
Nisin A	G	A	S	P	R		I	T	S	I	S
Nisin Z	G	A	S	P	R		I	T	S	I	S
Subtilin	K	I	T	P	Q		W	K	S	E	S
Epidermin	G	A	E	P	R		I	A	S	K	F
Gallidermin	G	A	E	P	R		I	A	S	K	F
Pep 5	E	L	E	P	Q		T	A	G	P	A
Epicidin 280	E	L	E	A	Q		S	L	G	P	A
Epilancin K7	D	L	E	P	Q		S	A	S	V	L

Figure 2. Comparison of cleavage sites of some widely studied type A1 lantibiotics.

hydrophilic and possess a high proportion of charged amino acids, some of which are highly conserved (e.g., the FNLDV box) and have a net negative or slightly positive charge. They also possess a conserved serine (position –6 (the start of the propeptide is position 1)) and proline (position –2). Mutations introduced to the -18 to -14 (FNLDV) region of nisin made the secretion or intracellular accumulation of nisin undetectable [75], and similar mutations in Pep5 also significantly decreased the production of Pep5 [76]. Both type AI and type AII lantibiotics also differ in the residues preceding the propeptide. Site directed mutagenesis at arginine (position –1) and alanine (position –4), but not at the conserved proline (position –2) in prenisin, strongly affected cleavage of the leader and resulted in the extracellular accumulation of unprocessed inactive nisin [75]. This demonstrated that cleavage of the leader sequence is not essential for translocation and that the mutated residues are probably involved in leader protease recognition.

Type AII leaders generally possess highly negative net charges and have consensus sequences unlike those found in the type AI group (e.g., ELS/EVS and EL/EM [77]). These leaders are also more similar to the class II bacteriocin leader sequences as they contain a double glycine 'GG, GA or GS' motif immediately preceding the cleavage site (e.g., cytolysin [78] and lacticin 481 [79]). Because of this similarity, it is thought that the leaders are cleaved by peptidases with similar specificities [77] and that the LanM enzymes are present in this group of lantibiotics rather than LanB/C because of the difference in their leader sequences [3].

One function proposed for the leader sequences is protection of the producer strain against high concentrations of intracellular bacteriocin, as lantibiotics remain inactive while the leader peptide is attached [75]. Alternatively, the leader either may be involved as a recognition signal for the modification enzymes and transport proteins, or may help ensure a suitable conformation of the propeptide for its interaction with the processing enzymes [76].

Much work has been done to clarify the role of the different parts of the genetic loci required for lantibiotic production. The data obtained in these studies are now invaluable in programmes being designed to enhance productivity through bioproduction of lantibiotics. Immobilised enzyme complexes are able to introduce modifications into genetically engineered peptides. These approaches may be the key in the future, if lantibiotics are to be produced in a cost-effective manner, though such approaches are currently not routine and do not yet represent a less expensive alternative to fermentation in most cases.

2.4 Lantibiotics exert their primary actions at the cytoplasmic membrane of susceptible bacteria

Lantibiotics are generally only active against Gram-positive bacteria because they exert their primary action at the cytoplasmic membrane and the outer membrane of Gram-negative bacteria is impenetrable to these peptides [47]. A small number of reports of activity against Gram-negative bacteria have been made [80].

It is acknowledged that the main target in the cell wall for lantibiotics is the membrane bound precursor lipid II, which results in compromised peptidoglycan synthesis. This binding leads to the sequestration of lipid II blocking the actions of the transglycosylase and transpeptidase enzymes that crosslink the glycan chains of the cell wall [81]. A conserved sequence motif of one complete thioether ring system appears to be necessary for this to occur [82].

The two groups of lantibiotics defined by Jung and Sahl [46] differ significantly in their mechanisms of action. The type A

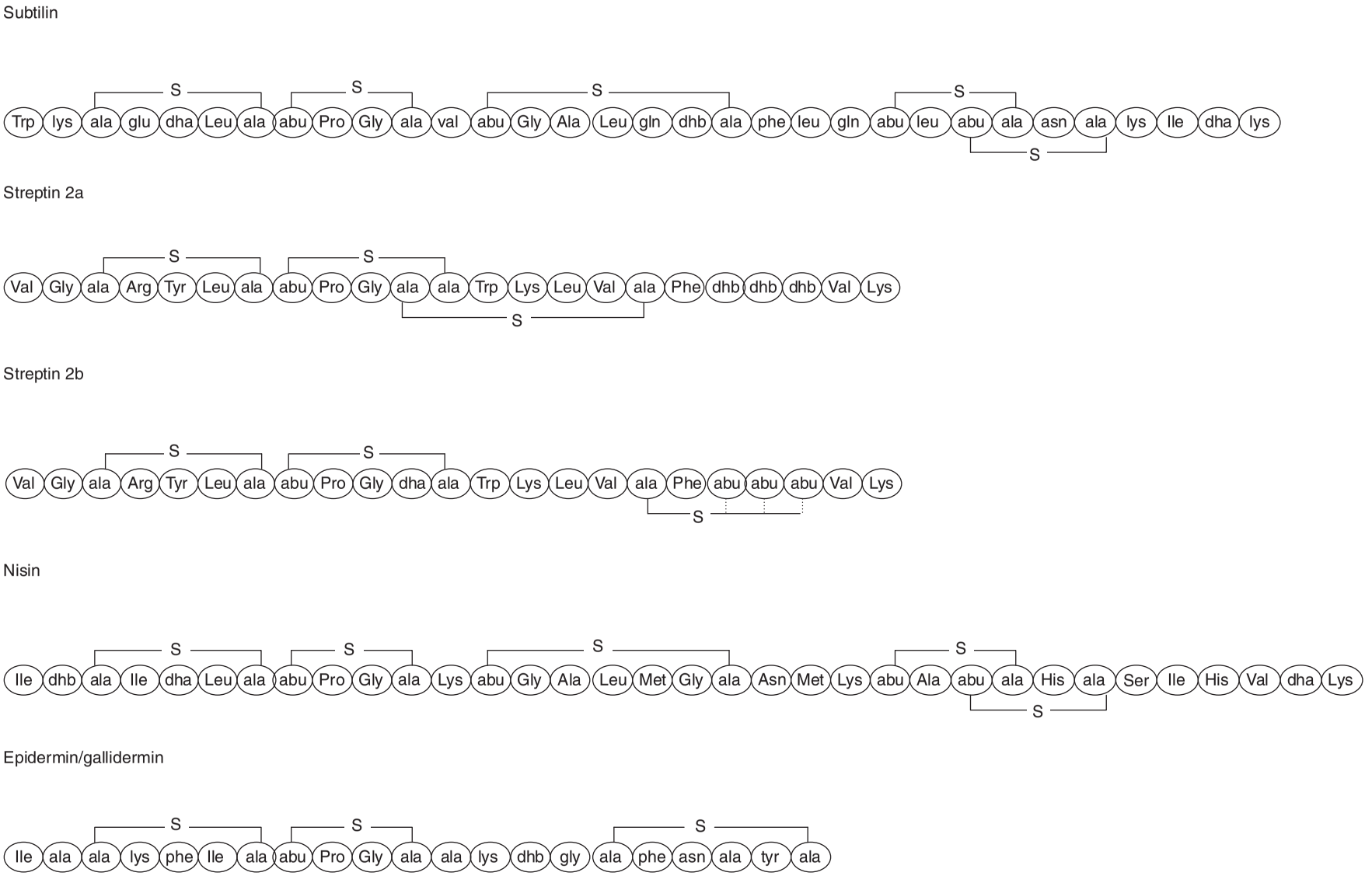

Figure 3. Example of the structure of propeptides of different lantibiotics. The -S- indicates the thioether cross linkages.

lantibiotics act through the formation of pores in the cytoplasmic membrane, leading to the loss of intracellular components and immediate cell death [47]. Nisin (type A) causes biosynthetic processes in susceptible cells to stop almost instantly [83]. Black lipid membrane studies demonstrated that nisin caused the formation of nonselective, voltage-dependent, short-lived transmembrane pores in the membrane [84]. In contrast, type B lantibiotics, while also acting at the cytoplasmic membrane, tend to bind to, and form complexes with, specific integral membrane components inactivating or inhibiting their function [47].

NMR-based structural analysis of nisin, in the presence of membrane mimicking micelles, indicated that the hydrophilic groups of the peptides interact with the phospholipid head groups, while the hydrophobic side chains were immersed in the hydrophobic core of the membrane bilayer, so that the molecules adopted a rod-like conformation [85,86]. These experiments gave rise to a model of action for pore formation as follows [87]. The ionic forces are responsible for the attraction between the positively-charged peptide side chains and the phospholipid head groups of the target membrane. Because the peptides are too small, and the attraction will not be sufficient to span the bilayer more than once, it has been suggested that several molecules preaggregate for pore formation [47]. As a result of the wedge-like insertion into the membrane, the peptides remain surface bound, which in turn would lead to local perturbation that finally creates short-lived pores [47].

This model, however, does not correlate with the activity data for type A lantibiotics *in vivo*. Much higher concentrations of peptide are necessary for pore formation in model membrane systems than for killing *in vitro*. There are also significant differences in the sensitivity of bacteria to these lantibiotics even in closely related strains [47]. Linnet and Strominger [88] showed that nisin could inhibit cell wall biosynthesis in *in vitro* systems, and this has since been shown to be due to the binding of nisin to the cell wall precursors, lipid-I and -II [89]. Epidermin and nisin are the only type A lantibiotics demonstrated to bind to lipid-I and -II to date. Pep5 has been shown to have no affinity for either, and thus may bind another (as yet unidentified) membrane molecule [82].

The N-terminal part of nisin was shown to be necessary for the initial binding to lipid-II. Mutations affecting the conformation of the N-terminal rings (e.g., S3T) reduced the affinity of nisin for lipid-II and the ability to block peptidoglycan biosynthesis, and prevented pore formation [90]. However, mutations affecting the flexible hinge region resulted in the peptides having no pore-forming activity at all. Interestingly, despite the loss of pore-forming ability, the peptides were reduced only a little in their *in vitro* activity [90]. This demonstrates that nisin has two distinct killing mechanisms: inhibition of cell wall biosynthesis at low concentrations and pore formation in the cytoplasmic membrane, leading to subsequent 'starvation' of the cell and death at higher concentrations. Pore formation probably overrides the slow lytic effect caused by peptidoglycan biosynthesis inhibition, but the latter effect may be a backup system important for the killing of cells that survive pore formation [47] or has a more significant role in situations of low peptide concentration.

Recent work has shown that interaction with lipid-II, leading to inhibition of cell wall construction, is exhibited by non-lanthionine containing peptides and the role of lipid-II in mode of action of these agents may, perhaps, be a more general phenomenon of cationic peptides, whether they are modified or not [91,92].

Mutacin II appears to have a completely different mode of action to that of any of the other lantibiotics described so far. It has been shown that mutacin II completely dissipates the transmembrane electrical potential and pH gradient of sensitive cells and causes only partial release of amino acids [1-^{14}C]2-α-aminoisobutyric acid (a non-metabolisable analogue of alanine) and glutamate [93]. This indicates that the mode of action of mutacin II may not rely on pore formation. A dramatic depletion of cellular ATP levels was observed. It was proposed that the partial depolarisation of the proton motive force was due to the permeation of the molecules across the cytoplasmic membrane to gain access to the cytosol. Once in the cytosol the molecule can interact with its primary target, which may be an enzyme involved in the generation of ATP at the substrate level [93].

The observation that several lantibiotics, including nisin and haloduracin, inhibit spore outgrowth [70,94] may be directly linked to membrane damage in germinating spores [95]. The maximum level of haloduracin activity against Gram-positive bacteria and *Bacillus anthracis* spores is seen at a ratio of 1:1 for the two-component peptides [96]. Halα initially binds to a target on the bacterial cell surface and Halβ leads to pore formation and potassium efflux.

Pore formation is used by nisin [97] and mersacidin [82] and can manifest in combination with the inhibition of peptidoglycan synthesis by some lantibiotics [8]. Defined, stable pore formation is a consequence of the utilisation of lipid-II as a docking molecule by lantibiotics, which results in membrane damage, depolarisation and eventually the death of cell [98].

Hasper *et al.* have described an alternative mode of action in which lipid-II of Gram-positive bacteria is removed from the septum (the cell division site), thus, blocking cell wall synthesis [99].

Understanding the mode of action of different lantibiotics and the importance of different domains in key proteins will have an impact on engineering strategies for production of lantibiotics and, possibly, chimeras with modified or extended activity spectra. These data will also inform decisions about the most appropriate indications for certain lantibiotics for therapeutic use.

2.5 Bioproduction of lantibiotics

Given the sometimes problematic fermentation of lantibiotics, which has hampered exploitation to an extent, there is a

growing interest in development of bio-catalysis pathways for *in vitro* production of 'artificial' lantibiotics [99,100]. This field benefits from recent advances in genome sequencing, bioinformatic analysis and biosynthetic pathway manipulation.

Haloduracin is produced as two post-translationally modified peptides, Halα and Halβ by *Bacillus halodurans* C-125 [39,40,96]. Expressed and purified precursor peptides (HalA1 and HalA2) can be processed *in vitro* by purified modification enzymes HalM1 and HalM2, respectively, in what is a reconstruction of the biosynthetic pathway for a two-component lantibiotic [40].

More recently, *in vitro*-reconstituted lacticin 481 synthetase (LctM) was used in combination with synthetic peptide substrates containing Ser, Thr and Cys analogues to generate 11 analogues of lacticin 481 [100]. In addition to allowing examination of the substrate specificity of the synthetase enzymes, it was also observed that a number of the analogues generated exhibited higher levels of antibacterial activity than the parent compound [100]. The LctA analogues were produced using a combination of solid-phase peptide synthesis and expressed protein ligation. It is suggested that such approaches could be used for other ribosomally synthesised and post-translationally modified natural products including the microcins and patellamides [101,102].

The nisin modifying enzyme NisC has also been reconstituted *in vitro* allowing examination of the cyclisation process. The crystal structure of the reconstituted protein was also determined revealing similarities in fold and substrate activation with mammalian farnesyl transferases. This interesting observation indicates that human homologues of NisC may be involved in modification of a peptide or protein substrate [103].

It is also worth noting that recent advances in modification and bioengineering have allowed the production of efficacious lantibiotics that have proven otherwise difficult to obtain, or were identified solely through data mining exercises. A key example is the recent use of the nisin synthetase machinery, which includes the modification enzymes NisB, NisC and NisT, for production of novel streptococcal lantibiotics [104]. Such approaches are proving more adaptable than previously would have been expected and will have a significant impact in future discovery programmes.

2.6 Lantibiotics have potential as therapeutic agents

The most extensive practical application of lantibiotics to date has been that of nisin, which is now used as a food preservative in > 50 countries [105]. Its proteinaceous nature and lack of toxicity, as measured when used non-systemically, makes nisin ideally suited for use in the food industry, where its broad spectrum activity allows control of many important Gram-positive and endospore-forming food pathogens. More recently, it has been suggested that nisin may also be useful in therapy of peptic ulcers caused by *Helicobacter pylori* [105]. This is due to its stability in acidic conditions and insensitivity to pepsin, which allows it to maintain antimicrobial activity in the stomach. The degradation of nisin on exposure to pancreatic enzymes on the other hand means the intestinal flora is not affected by it [105].

The minimum inhibitory concentrations of mutacin B-Ny266 and nisin A have been compared with those of vancomycin and oxacillin against various bacterial pathogens [80]. In addition, both lantibiotics were found to be active against strains of *Neisseria* species, making them good candidates for use against *Neisseria* infections. Mutacin B-Ny266 was also found to be active against *H. pylori* strains, thus, making it a potential option to replace conventional antibiotics.

Adsorption of nisin onto silica surfaces has been shown to suppress the colonisation of *Listeria monocytogenes* onto these surfaces [106]. This indicates that the adsorption of nisin to food contact surfaces may have the potential to prevent unwanted colonisation of pathogenic organisms, such as *L. monocytogenes*.

The direct application of lantibiotic-producing bacteria as fermenters either in food production (as in the case for nisin) or in normal flora replacement therapy in humans and other animals may also be a way to broaden their use. For example, nisin has proven to be effective in the treatment of bovine clinical mastitis, mainly those infections caused by staphylococci [107].

Although lantibiotics have not yet been used in the setting of clinical chemotherapy on the same scale as conventional antibiotics, they have already been examined in preclinical phase trials. Some of the following companies have been leading in the development of cationic peptides for clinical use and their websites are a source of regularly updated information.

A number of patents have been filed claiming utility of lantibiotics in a range of indications. Novacta Biosystems Ltd is developing a type-B lantibiotic-based compound that has antibacterial activity (PCT/GB2008/002463). The compound is proposed for use in therapy or prophylaxis for infections caused by *Clostridium difficile* and for treatment of pseudomembranous colitis.

The agent is administered orally and survives passage through the stomach, thus, displaying an increased stability to enzymatic degradation compared to nisin. This is probably a result of the globular structure. As the spectrum of activity of lantibiotics can be narrow, it has been suggested that the agent in development by Novacta Biosystems may have reduced activity against commensal gut flora (e.g., *Bifidobacterium* species and *Bacteroides* species), compared to vancomycin or metronidazole. Clearly, this is an advantage for treatment of *C. difficile* infection.

Molchem Medicines, Inc. has incorporated at least one lantibiotic into a pharmaceutical preparation for the treatment of membrane-associated diseases and disorders (PCT/US2005/015901).

The lantibiotic nisin has been shown to act synergistically with mupirocin and gentamicin for treating bacterial infections by Biosynexus, Inc., who have suggested that

combinations of a variety of lantibiotics including subtilin, epidermin, gallidermin, pep 5, cinnamycin, duramycin or ancovenin may have use in this respect (PCT/US2006/039608). It is proposed that these pharmaceutical compositions could be formulated as a cream, spray, used in timed-release formulations or solutions (e.g., colloidal solution), mixed with fibrin glue, impregnated onto a wound dressing or bandage, or formulated into a delivery vehicle such as liposomes.

Clearly, a number of attempts to exploit lantibiotics have been described, but we now need to capitalise on previous advances and make good use of recent innovations to fully realise the potential of some lantibiotics for use in the medical field.

3. Expert opinion

The dawn of the post-antibiotic era has been heralded for several years, but until very recently we have been spared the spectre of 'un-treatable' infections. The description of New Delhi metallo-β-lactamase-1 producing *Klebsiella* isolates [108] is probably the closest we have come to bacteria that are resistant to all antibiotics. This is as clear a warning we are likely to get that new therapeutics is urgently required.

The control of infectious diseases is now based on the selection and careful use of a large group of low-molecular-mass inhibitors with diverse mechanisms of action and various spectra of antibacterial and antifungal activities [80]. Microorganisms, however, have developed a variety of defence mechanisms against antibiotics and antibacterial drug resistance has been reported for most of the predominant pathogenic bacteria [13,109]. Amongst the most promising antibacterial agents being investigated as useful replacement therapeutics are antibacterial peptides, such as the lantibiotics [13].

Conventional approaches to discovery of lantibiotics for medical application have centred on large programmes involving labour intensive screening for inhibitory activity followed by chromatographic purification, amino-acid sequence determination and time consuming reverse genetic approaches. Recent advances in genetics and peptide engineering offer more efficient and cost effective means of accessing the potentially useful novel antibiotics in bacteria.

The phenomenal developments in generation of DNA sequence data are already proving their utility in the field of bacteriocin discovery. Using draft genome sequence determination, our group has recently been able to progress from amino-acid sequence tag data to identification of the genetic apparatus required for production of several novel agents in a matter of 2 weeks (unpublished data); this process would have taken a minimum of several months using reverse genetic approaches. The costs we incurred for draft genome sequence determination were probably equivalent to those for reverse genetic methods, especially if investigator time was accounted for.

It has also been recently suggested that increases in performance of the next-generation sequencing platforms, with longer read and contig lengths, will allow access to data relating to bacteriocin genetics in the outputs from metagenomic studies [45], many of which are becoming publicly available.

Further use of lantibiotics may depend greatly on the success of peptide engineering to create variant structures. Increasing the stability of presently-known lantibiotics may also broaden their applicability by reducing their sensitivity to increased temperature and pH extremes, increasing their solubility in water and increasing their inhibitory range. The improvement of some of these characteristics has already been demonstrated for nisin Z, where the solubility in water was increased by the substitution of lysine for the amino acids in positions 27 or 31, without altering the antimicrobial activity and inhibitory spectrum of the molecule [110]. Alterations such as these may allow a wider range of lantibiotics to be used in the food industry [71].

Another potential therapeutic benefit of certain lantibiotics may include immunoregulation. The cinnamycin subtype of type B lantibiotics has been shown to bind specifically to phosphatidylethanolamine resulting in the inhibition of phospholipase A2, an important enzyme involved in the biosynthesis of prostaglandins and leucotrienes [111].

As cationic antibacterial peptides, lantibiotics are considered to be anti-infective agents, as they have potential as effectors or have a role in innate immunity and could upregulate the expression of multiple genes in eukaryotic cells [111,112]. It has been suggested that cationic peptides have a role in modulation of the immune system as their presence leads to suppression of the eukaryotic cellular response to bacterial molecules such as lipopolysaccharide and lipoteichoic acid [112].

The potential applications for lantibiotics that are described above more than justify continued research into the identification of new bacteriocins. By increasing our understanding of the various mechanisms of antibacterial action and working to expand the range of applications for these agents, we will be well placed to maximise the benefit that these peptides may provide. The increasing availability of public genome and meta-genome data and the ease with which draft genome data can be generated for a lab strain of interest will have a huge impact in the near future. If we can sensibly combine bioinformatics, genetic and peptide engineering approaches, developmental lantibiotics may have significant benefit for therapy of infectious diseases in these difficult times.

Declaration of interest

MU is a Higher Education Funding Council for England staff member. M Al-Mahrous was supported by the Saudi Arabian Health Ministry.

Bibliography

1. Dunstan G, Avignone-Rossa C, Langley D, Bushell M. The Vancomycin biosynthetic pathway is induced in oxygen-limited Amycolatopsis orientalis (ATCC 19795) cultures that do not produce antibiotic. Enzyme Microbiol Technol 2000;27(7):502-10
2. Jack R, Sahl H, Bierbaum G. Lantibiotics and related peptides. Biotechnology intelligence unit. Springer-Verlag, Berlin; 1998. p. 224p
3. Jack R, Tagg J, Ray B. Bacteriocins of Gram-positive bacteria. Microbiol Rev 1995;59(2):171-200
4. Projan S, Shlaes D. Antibacterial drug discovery: is it all downhill from here? Clin Microbiol Infect 2004;10(s4):18-22
5. Bush K. Antibacterial drug discovery in the 21st century. Clin Microbiol Infect 2004;(10 Suppl 4):10-17
6. Shlaes DM. The abandonment of antibacterials: why and wherefore? Curr Opin Pharmacol 2003;3(5):470-3
7. Overbye K, Barrett J. Antibiotics: where did we go wrong? Drug Discov Today 2005;10(1):45-52
8. Breukink E, de Kruijff B. Lipid-II as a target for antibiotics. Nat Rev Drug Discov 2006;5(4):321-32
9. Cotter PD, Hill C, Ross RP. Bacterial lantibiotics: strategies to improve therapeutic potential. Curr Protein Pept Sci 2005;6(1):61-75
10. Willey J, van der Donk W. Lantibiotics: peptides of diverse structure and function. Annu Rev Microbiol 2007;61:477-501
11. Enserink M. Promising antibiotic candidate identified. Science 1999;286(5448):2245-7
12. Field D, Hill C, Cotter PD, Ross RP. The dawning of a 'Golden era' in lantibiotic bioengineering. Mol Microbiol 2010;78(5):1077-87
13. Sahl H, Bierbaum G. Lantibiotics: biosynthesis and biological activities of uniquely modified peptides from Gram-positive bacteria. Annu Rev Microbiol 1998;52:41-79
14. Kodani S, Lodato MA, Durrant MC, et al. SapT, a lanthionine-containing peptide involved in aerial hyphae formation in the streptomycetes. Mol Microbiol 2005;58(5):1368-80
15. Sahl H, Jack R, Bierbaum G. Biosynthesis and biological activities of lantibiotics with unique post-translational modifications. Eur J Biochem 1995;230(3):827-53
16. Heng N, Wescombe P, Burton J, et al. The diversity of bacteriocins in Gram-positive bacteria, in Bacteriocins. Springer Berlin Heidelberg, Berlin; 2007. p. 45-92
17. Klaenhammer T. Genetics of bacteriocins produced by lactic acid bacteria. FEMS Microbiol Rev 1993;12(1-3):39-85
18. Jack R, Bierbaum G, Heidrich C, Sahl H. The genetics of lantibiotic biosynthesis. Bioessays 1995;17(9):793-802
19. Schenk S, Laddaga RA. Improved method for electroporation of Staphylococcus aureus. FEMS Microbiol Lett 1992;73(1-2):133-8
20. van de Kamp M, van den Hooven H, Konings R, et al. Elucidation of the primary structure of the lantibiotic epilancin K7 from Staphylococcus epidermidis K7: cloning and characterisation of the epilancin-K7-encoding gene and NMR analysis of mature epilancin K7. Eur J Biochem 1995;230(2):587-600
21. Cotter P, et al. Overproduction of wild-type and bioengineered derivatives of the lantibiotic lacticin 3147. Appl Environ Microbiol 2006;72(6):4492-6
22. Bierbaum G, et al. The biosynthesis of the lantibiotics epidermin, gallidermin, Pep5 and epilancin K7. Antonie Van Leeuwenhoek 1996;69(2):119-27
23. Xie L, Miller LM, Chatterjee C, et al. Lacticin 481: in vitro reconstitution of lantibiotic synthetase activity. Science 2004;303(5658):679-81
24. Page C. In vitro synthesis of lantibiotics. Science 2004;303(5658):585g
25. Lee MV, Ihnken LA, You YO, et al. Distributive and directional behavior of lantibiotic synthetases revealed by high-resolution tandem mass spectrometry. J Am Chem Soc 2009;131(34):12258-64
26. Moll GN, Kuipers A, Rink R. Microbial engineering of dehydro-amino acids and lanthionines in non-lantibiotic peptides. Antonie Van Leeuwenhoek 2010;97(4):319-33
27. Engelke G, Gutowski-Eckel Z, Hammelmann M, Entian K. Regulation of nisin biosynthesis and immunity in Lactococcus lactis 6F3. Appl Environ Microbiol 1994;60(3):814-25
28. Klein C, Kaletta C, Entian K. Biosynthesis of the lantibiotic subtilin is regulated by a histidine kinase/response regulator system. Appl Environ Microbiol 1993;59(1):296-303
29. McLaughlin R, Ferretti J, Hynes W. Nucleotide sequence of the streptococcin A-FF22 lantibiotic regulon: model for production of the lantibiotic SA-FF22 by strains of Streptococcus pyogenes. FEMS Microbiol Lett 1999;175(2):171-7
30. Upton M, Tagg J, Wescombe P, Jenkinson H. Intra- and inter-species signaling between Streptococcus salivarius and Streptococcus pyogenes mediated by SalA and SalA1 lantibiotic peptides. J Bacteriol 2001;183(13):3931-8
31. Altena K, Guder A, Cramer C, Bierbaum G. Biosynthesis of the lantibiotic mersacidin: organization of a type B lantibiotic gene cluster. Appl Environ Microbiol 2000;66(6):2565-71
32. Schnell N, Engelke G, Augustin J, et al. Analysis of genes involved in the biosynthesis of lantibiotic epidermin. Eur J Biochem 1992;204(1):57-68
33. McAuliffe O, Ross R, Hill C. Lantibiotics: structure, biosynthesis and mode of action. FEMS Microbiol Rev 2001;25(3):285-308
34. Siegers K, Entian K. Genes involved in immunity to the lantibiotic nisin produced by Lactococcus lactis 6F3. Appl Environ Microbiol 1995;61(3):1082-9
35. Peschel A, Gotz F. Analysis of the Staphylococcus epidermidis genes epiF, -E, and -G involved in

epidermin immunity. J Bacteriol 1996;178(2):531-6

36. Rince A, Dufour A, Uguen P, et al. Characterization of the lacticin 481 operon: the Lactococcus lactis genes lctF, lctE, and lctG encode a putative ABC transporter involved in bacteriocin immunity. Appl Environ Microbiol 1997;63(11):4252-60

37. Otto M, Submuth R, Jung G, Gotz F. Structure of the pheromone peptide of the Staphylococcus epidermidis agr system. FEBS Lett 1998;424(1-2):89-94

38. Hyink O, Balakrishnan M, Tagg J. Streptococcus rattus strain BHT produces both a class I two-component lantibiotic and a class II bacteriocin. FEMS Microbiol Lett 2005;252(2):235-41

39. Lawton EM, Cotter PD, Hill C, Ross RP. Identification of a novel two-peptide lantibiotic, haloduracin, produced by the alkaliphile Bacillus halodurans C-125. FEMS Microbiol Lett 2007;267(1):64-71

40. McClerren AL, Cooper LE, Quan C, et al. Discovery and in vitro biosynthesis of haloduracin, a two-component lantibiotic. Proc Natl Acad Sci USA 2006;103(46):17243-8

41. Begley M, Cotter P, Hill C, Ross R. Identification of a novel two-peptide lantibiotic, lichenicidin, following rational genome mining for LanM proteins. Appl Environ Microbiol 2009;75(17):5451-60

42. Banerjee S, Hansen J. Structure and expression of a gene encoding the precursor of subtilin, a small protein antibiotic. J Biol Chem 1988;263(19):9508-14

43. Rodriguez J, Dodd H. Genetic determinants for the biosynthesis of nisin, a bacteriocin produced by Lactococcus lactis. Microbiologia 1996;12(1):61-74

44. Rauch P, Beerthuyzen M, de Vos W. Distribution and evolution of nisin-sucrose elements in Lactococcus lactis. Appl Environ Microbiol 1994;60(6):1798-804

45. de Jong A, van Heel AJ, Kok J, Kuipers OP. BAGEL2: mining for bacteriocins in genomic data. Nucl Acids Res 2010;38(Suppl 2):W647-651

46. Jung G, Sahl H. Nisin and novel lantibiotics. In:Jung G, Sahl H, editors, Proceedings of the 1st International Workshop on Lantibiotics. Escom, Leiden; 1991. p. 490

47. Guder A, Wiedemann I, Sahl H. Post-translationally modified bacteriocins: the lantibiotics. Curr Protein Pept Sci 2000;55(1):62-73

48. Uguen P, Le Pennec J, Dufour A. Lantibiotic biosynthesis: interactions between prelacticin 481 and its putative modification enzyme, LctM. J Bacteriol 2000;182(18):5262-6

49. Gilmore M, Segarra R, Booth M, et al. Genetic structure of the Enterococcus faecalis plasmid pAD1-encoded cytolytic toxin system and its relationship to lantibiotic determinants. J Bacteriol 1994;176(23):7335-44

50. Navaratna M, Sahl H, Tagg J. Two-component anti-Staphylococcus aureus lantibiotic activity produced by Staphylococcus aureus C55. Appl Environ Microbiol 1998;64(12):4803-8

51. Ryan M, Jack R, Josten M, et al. Extensive post-translational modification, including serine to D-alanine conversion, in the two-component lantibiotic, lacticin 3147. J Biol Chem 1999;274(53):37544-50

52. Koponen O, Tolonen M, Qiao M, et al. NisB is required for the dehydration and NisC for the lanthionine formation in the post-translational modification of nisin. Microbiology 2002;148(11):3561-8

53. Pag U, Sahl HG. Multiple activities in lantibiotics–models for the design of novel antibiotics? Curr Pharm Des 2002;8(9):815-33

54. Goto Y, Li B, Claesen J, et al. Discovery of unique lanthionine synthetases reveals new mechanistic and evolutionary insights. PLoS Biol 2010;8(3):e1000339

55. Li B, Sher D, Kelly L, et al. Catalytic promiscuity in the biosynthesis of cyclic peptide secondary metabolites in planktonic marine cyanobacteria. Proc Natl Acad Sci USA 2010;107(23):10430-5

56. Christianson DW. Biochemistry. Five golden rings. Science 2006;311(5766):1382-3

57. Ferretti J, McShan W, Ajdic D, et al. Complete genome sequence of an M1 strain of Streptococcus pyogenes. Proc Natl Acad Sci USA 2001;98(8):4658-63

58. Wescombe P, Tagg J. Purification and characterization of streptin, a type A1 lantibiotic produced by Streptococcus pyogenes. Appl Environ Microbiol 2003;69(5):2737-47

59. Immonen T, Ye S, Ra R, et al. The codon usage of the nisZ operon in Lactococcus lactis N8 suggests a non-lactococcal origin of the conjugative nisin-sucrose transposon. DNA Seq 1995;5(4):203-18

60. Kunst F, Ogasawara N, Moszer I, et al. The complete genome sequence of the gram-positive bacterium Bacillus subtilis. Nature 1997;390(6657):249-56

61. Karaya K, Shimizu T, Taketo A. New gene cluster for lantibiotic streptin possibly involved in streptolysin S formation. J Biochem 2001;129(5):769-75

62. Schnell N, Entian K-D, Schneider U, et al. Prepeptide sequence of epidermin, a ribosomally synthesized antibiotic with four sulphide-rings. Nature 1988;333(6170):276-8

63. Skaugen M, Nissen-Meyer J, Jung G, et al. In vivo conversion of L-serine to D-alanine in a ribosomally synthesized polypeptide. J Biol Chem 1994;269(44):27183-5

64. Heidrich C, Pag U, Josten M, et al. Isolation, characterization, and heterologous expression of the novel lantibiotic epicidin 280 and analysis of its biosynthetic gene cluster. Appl Environ Microbiol 1998;64(9):3140-6

65. Kuipers O, Rollema H, Yap W, et al. Engineering dehydrated amino acid residues in the antimicrobial peptide nisin. J Biol Chem 1992;267(34):24340-6

66. Rayman M, Aris B, Hurst A. Nisin: a possible alternative or adjunct to nitrite in the preservation of meats. Appl Environ Microbiol 1981;41(2):375-80

67. Kleinkauf H, Von Dohren H. Biosynthesis of peptide antibiotics. Annu Rev Microbiol 1987;41(1):259-89

68. Liu W, Hansen J. Some chemical and physical properties of nisin, a small-protein antibiotic produced by Lactococcus lactis.

Appl Environ Microbiol 1990;56(8):2551-8

69. van Kraaij C, Breukink E, Rollema H, et al. Engineering a disulfide bond and free thiols in the lantibiotic nisin Z. Eur J Biochem 2000;267(3):901-9

70. Chan W, Leyland M, Clark J, et al. Structure-activity relationships in the peptide antibiotic nisin: antibacterial activity of fragments of nisin. FEBS Lett 1996;390(2):129-32

71. Liu W, Hansen J. Enhancement of the chemical and antimicrobial properties of subtilin by site-directed mutagenesis. J Biol Chem 1992;267(35):25078-85

72. van der Meer J, Polman J, Beerthuyzen M, et al. Characterization of the Lactococcus lactis nisin A operon genes nisP, encoding a subtilisin-like serine protease involved in precursor processing, and nisR, encoding a regulatory protein involved in nisin biosynthesis. J Bacteriol 1993;175(9):2578-88

73. Weil H, Beck-Sickinger A, Metzger J, et al. Biosynthesis of the lantibiotic Pep5: isolation and characterization of a prepeptide containing dehydroamino acids. Eur J Biochem 1990;194(1):217-23

74. Izard J, Kendall D. Signal peptides: exquisitely designed transport promoters. Mol Microbiol 1994;13(5):765-73

75. van der Meer J, Rollema H, Siezen R, et al. Influence of amino acid substitutions in the nisin leader peptide on biosynthesis and secretion of nisin by Lactococcus lactis. J Biol Chem 1994;269(5):3555-62

76. Neis S, Bierbaum G, Josten M, et al. Effect of leader peptide mutations on biosynthesis of the lantibiotic Pep5. FEMS Microbiol Lett 1997;149(2):249-55

77. de Vos W, Kuipers O, van der Meer J, Siezen R. Maturation pathway of nisin and other lantibiotics: post-translationally modified antimicrobial peptides exported by Gram-positive bacteria. Mol Microbiol 1995;17(3):427-37

78. Gilmore M, Segarra R, Booth M. An HlyB-type function is required for expression of the Enterococcus faecalis hemolysin/bacteriocin. Infect Immun 1990;58(12):3914-23

79. Piard J, Kuipers O, Rollema H, et al. Structure, organization, and expression of the lct gene for lacticin 481, a novel lantibiotic produced by Lactococcus lactis. J Biol Chem 1993;268(22):16361-8

80. Morency H, Mota-Meira M, LaPointe G, et al. Comparison of the activity spectra against pathogens of bacterial strains producing a mutacin or a lantibiotic. Can J Microbiol 2001;47(4):322-31

81. Lazar K, Walker S. Substrate analogues to study cell-wall biosynthesis and its inhibition. Curr Opin Chem Biol 2002;6(6):786-93

82. Brotz H, Bierbaum G, Leopold K, et al. The lantibiotic mersacidin inhibits peptidoglycan synthesis by targeting lipid-II. Antimicrob Agents Chemother 1998;42(1):154-60

83. Brotz H, Bierbaum G, Markus A, et al. Mode of action of the lantibiotic mersacidin: inhibition of peptidoglycan biosynthesis via a novel mechanism? Antimicrob Agents Chemother 1995;39(3):714-19

84. Wiedemann I, Benz R, Sahl H. Lipid-II-mediated pore formation by the peptide antibiotic nisin: a black lipid membrane study. J Bacteriol 2004;186(10):3259-61

85. Hooven H, Doeland C, Kamp M, et al. Three-dimensional structure of the lantibiotic nisin in the presence of membrane-mimetic micelles of dodecylphosphocholine and of sodium dodecylsulphate. Eur J Biochem 1996;235:382-93

86. Van Den Hooven HW, Spronk CA, Van De Kamp M, et al. Surface location and orientation of the lantibiotic nisin bound to membrane-mimicking micelles of dodecylphosphocholine and of sodium dodecylsulphate. Eur J Biochem 1996;235:394-403

87. Kuipers A, de Boef E, Rink R, et al. NisT, the transporter of the lantibiotic nisin, can transport fully modified, dehydrated and unmodified prenisin and fusions of the leader peptide with non-lantibiotic peptides. J Biol Chem 2004;279(21):22176-82

88. Linnett P, Strominger J. Additional antibiotic inhibitors of peptidoglycan synthesis. Antimicrob Agents Chemother 1973;4(3):231-6

89. Reisinger P, Seidel H, Tschesche H, Hammes WP. The effect of nisin on murein synthesis. Arch Microbiol 1980;127(3):187-93

90. Wiedemann I, Breukink E, van Kraaij C, et al. Specific binding of nisin to the peptidoglycan precursor lipid-II combines pore formation and inhibition of cell wall biosynthesis for potent antibiotic activity. J Biol Chem 2001;276(3):1772-9

91. Schneider T, Kruse T, Wimmer R, et al. Plectasin, a fungal defensin, targets the bacterial cell wall precursor Lipid II. Science 2010;328(5982):1168-72

92. Fan JA, Wu C, Bao K, et al. Self-assembled plasmonic nanoparticle clusters. Science 2010;328(5982):1135-8

93. Venema K, Chikindas ML, Seegers J, et al. Rapid and efficient purification method for small, hydrophobic, cationic bacteriocins: purification of lactococcin B and pediocin PA-1. Appl Environ Microbiol 1997;63(1):305-9

94. Campbell LL Jr, Sniff EE. Effect of subtilin and nisin on the spores of Bacillus coagulans. J Bacteriol 1959;77(6):766-70

95. Gut IM, Prouty AM, Ballard JD, et al. Inhibition of Bacillus anthracis spore outgrowth by nisin. Antimicrob Agents Chemother 2008;52(12):4281-8

96. Oman TJ, van der Donk WA. Insights into the mode of action of the two-peptide lantibiotic haloduracin. ACS Chem Biol 2009;4(10):865-74

97. Breukink E, Wiedemann I, van Kraaij C, et al. Use of the cell wall precursor lipid-II by a pore-forming peptide antibiotic. Science 1999;286(5448):2361-4

98. Brotz H, Josten M, Wiedemann I, et al. Role of lipid-bound peptidoglycan precursors in the formation of pores by nisin, epidermin and other lantibiotics. Mol Microbiol 1998;30(2):317-27

99. Hasper HE, Kramer NE, Smith JL, et al. An alternative bactericidal mechanism of action for lantibiotic peptides that target lipid II. Science 2006;313(5793):1636-7

100. Levengood MR, Knerr PJ, Oman TJ, van der Donk WA. In vitro

mutasynthesis of lantibiotic analogues containing nonproteinogenic amino acids. J Am Chem Soc 2009;131(34):12024-5

101. Li JW, Vederas JC. Drug discovery and natural products: end of an era or an endless frontier? Science 2009;325(5937):161-5

102. Zhang X, van der Donk WA. Using expressed protein ligation to probe the substrate specificity of lantibiotic synthetases. Methods Enzymol 2009;462:117-34

103. Li B, Yu JP, Brunzelle JS, et al. Structure and mechanism of the lantibiotic cyclase involved in nisin biosynthesis. Science 2006;311(5766):1464-7

104. Majchrzykiewicz JA, et al. Production of a class II two-component lantibiotic of Streptococcus pneumoniae using the class I nisin synthetic machinery and leader sequence. Antimicrob Agents Chemother 2010;54(4):1498-505

105. Delves-Broughton J, Blackburn P, Evans RJ, Hugenholtz J. Applications of the bacteriocin, nisin. Antonie van Leeuwenhoek 1996;69(2):193-202

106. Bower C, McGuire J, Daeschel M. Suppression of Listeria monocytogenes colonization following adsorption of nisin onto silica surfaces. Appl Environ Microbiol 1995;61(3):992-7

107. Cao LT, Wu JQ, Xie F, et al. Efficacy of nisin in treatment of clinical mastitis in lactating dairy cows. J Dairy Sci 2007;90(8):3980-5

108. Kumarasamy KK, Toleman MA, Walsh TR, et al. Emergence of a new antibiotic resistance mechanism in India, Pakistan, and the UK: a molecular, biological, and epidemiological study. Lancet Infect Dis 2010;28(3):265-6

109. Al-Mahrous M, Sandiford SK, Tagg JR, Upton M. Purification and characterization of a novel delta-lysin variant that inhibits Staphylococcus aureus and has limited hemolytic activity. Peptides 2010;31(9):1661-8

110. Rollema HS, Kuipers OP, Both P, et al. Improvement of solubility and stability of the antimicrobial peptide nisin by protein engineering. Appl Environ Microbiol 1995;61(8):2873-8

111. Marki F, Li JW, Vederas JC. Mode of action of the lanthionine-containing peptide antibiotics duramycin, duramycin B and C, and cinnamycin as indirect inhibitors of phospholipase A2. Biochem Pharmacol 1991;42(10):2027-35

112. Hancock R. Cationic peptides: effectors in innate immunity and novel antimicrobials. Lancet Infect Dis 2001;1(3):156

Affiliation

Mohammed M Al-Mahrous & Mathew Upton[†]
[†]Author for correspondence
University of Manchester,
School of Translational Medicine,
Department of Medical Microbiology,
Clinical Sciences Building,
Manchester Royal Infirmary,
Oxford Road, Manchester,
M13 9WL, UK
Tel: +44 1 161 276 8828;
Fax: +44 0 161 276 8826;
E-mail: m.upton@manchester.ac.uk