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Antimicrobial peptides and bacteriocins: alternatives to traditional antibiotics

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Abstract

Antimicrobial peptides (AMPs) are ubiquitous, gene-encoded natural antibiotics that have gained recent attention in the search for new antimicrobials to combat infectious disease. In multicellular organisms, AMPs, such as defensins and cathelicidins, provide a coordinated protective response against infection and are a principal component of innate immunity in vertebrates. In unicellular organisms, AMPs, such as bacteriocins, function to suppress competitor species. Because many AMPs kill bacteria by disruption of membrane integrity and are thus thought to be less likely to induce resistance, AMPs are being extensively evaluated as novel antimicrobial drugs. This review summarizes and discusses the antibiotic properties of AMPs highlighting their potential as alternatives to conventional antibiotics.

Keywords: antibiotics, antimicrobial peptides, bacteriocin, animal health

Introduction

Antimicrobial peptides (AMPs): ubiquitous natural antibiotics

Small biological molecules (<10 kDa) with direct antimicrobial activity, including enzymatically synthesized compounds and ribosomal-synthesized AMPs, provide effective microbial defense for all organisms from bacteria to mammals (Beutler, 2004; Hancock and Sahl, 2006). The discovery and development of conventional antibiotics, which are primarily based on bacteria- or fungi-generated antimicrobial compounds, have led to dramatic improvements in the ability to treat infectious diseases and significant increases in food animal production. Unquestionably, antibiotics represent one of the major scientific and medical advances of the 20th century (Gordon *et al.*, 2005; McPhee and Hancock, 2005). Although antibiotic therapy is still the first choice to combat microbial infections in humans and animals, the prevalence of bacterial resistance to conventional antibiotics is a growing public health concern. This has driven the search for new antimicrobials that are broadly effective and less likely to induce antimicrobial resistance.

Natural gene-encoded AMPs are a diverse group of innate immune molecules present in all organisms. Mature AMPs generally contain 12–100 amino acid residues, possess a net positive charge and an amphipathic structure that facilitates interaction with negatively charged microbial membranes or other cellular targets (Yeaman and Yount, 2007; Linde *et al.*, 2008; Sang and Blecha, 2008). A list of the general properties of AMPs, including primary structural properties and antimicrobial activities, is presented in Table 1. Compared with conventional antibiotics, which are generally active against bacteria or fungi, AMPs often exert activity against a broad spectrum of micro-organisms including bacteria, fungi, parasites, enveloped viruses and even some cancer cells. In addition, unlike conventional antibiotics, which generally target a metabolic enzyme and may selectively induce resistance in micro-organisms, AMPs kill microbes mainly by membrane-targeting pore-forming mechanisms (Table 2), a mechanism that is inherently more difficult for microbes to circumvent by developing resistance (Boman, 2003; Hancock and Sahl, 2006). AMPs have been isolated from most life forms and include bacteriocins,

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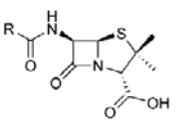
Table 1. Examples and general properties of AMPs

Organism	Class/subclass	Examples	Main structural properties	Antimicrobial activity
LAB[1]	Class I and II bacteriocins	Lantibiotics Class I: nisin, mersacidin; non-lantibiotics. Class II: pediocin, PA1, enterocin AS48	Class I: extensive post-translational residue modification. Either elongated cationic or globular conformation. Class II: very diverse group. Pediocin-like members conforming β-sheet structures and a C-terminal α-helix	Nanomolar range, active against closely related or broad-spectrum Gram-positive bacteria
Other bacteria (e.g. *E. coli*)[2]	Bacteriocins	Colicins, microcins	α-Helix-rich globular structure	Nanomolar range, active against Enterobacteriaceae
Fungi[3]	Fungal defensins	Plectasin	Cysteine-rich, containing two antiparallel β-sheets and an α-helix	MIC: 1–35 μg/ml for multiple antibiotic-resistant Gram-positive bacteria
Plants[4]	Plant defensins	Ib-AMP1–4 and cyclotides	Cysteine-rich, containing antiparallel β-sheets, and cyclotides with cyclic backbone and cysteine knot	Micromolar range: antifungal (Ib-AMPs), anti-HIV, anti-parasites (cyclotides)
Insects/ amphibians[5]	Insect/ amphibian cationic peptides	Cecropin A, mellitin, magainins, temporins	High basic residue content, form α-helix containing structures in membrane	Micromolar range active against multidrug-resistant bacteria
Arachnida/ vertebrates[6]	Venom toxins/ β-defensins	Defensin-like toxins (DLTs) in venom, and β-defensins	β-Sheets and disulfide bonds form into β-hairpins, and D-amino acid post-translational modification in DLTs	Micromolar range, active against multidrug-resistant bacteria mostly in a salt-dependent manner
Mammals[7]	α-Defensins θ-Defensins β-Defensins	Human neutrophil defensins, enteric and epithelial defensins	β-Sheets and disulfide bonds form into β-hairpins	Micromolar range active against multidrug-resistant bacteria, and fungi and viruses
Higher vertebrates[8]	Cathelicidins	Human LL-37, porcine PR-39, bovine indolicidin	Contain a cathelin leader sequence with an amphipathic cationic mature peptide	Micromolar range active against multidrug-resistant bacteria, and fungi and viruses
Humans[9]	Others	Lactoferricin, and antimicrobial domain of lysozyme	Derived anionic/cationic antimicrobial fragments from lactoferrin, casein and lysozyme	Micromolar range active against multidrug-resistant bacteria

[1]Willey *et al.*, 2007; Field *et al.*, 2008.
[2]Duquesne *et al.*, 2007; Nes *et al.*, 2007.
[3]Mygind *et al.*, 2005.
[4]Colgrave *et al.*, 2008; Ireland *et al.*, 2008; Marcos *et al.*, 2008.
[5]Bechinger, 1997; Giacometti *et al.*, 2003.
[6]Yeaman and Yount, 2007; Warren *et al.*, 2008.
[7]Selsted and Ouellette, 2005; Lehrer, 2007.
[8]Zanetti, 2005.
[9]Brogden, 2005.

fungal peptide antibiotics, plant thionins and defensins, insect defensins and cecropins, amphibian magainins and temporins, as well as defensins and cathelicidins from higher vertebrates (McPhee and Hancock, 2005; Yeaman and Yount, 2007). This review will summarize and discuss the antibiotic properties of AMPs with the aim of highlighting their potential as alternatives to conventional antibiotics.

Table 2. Characteristics and applications of biological antimicrobials

	Biological antimicrobials	
	Conventional antibiotics Enzymatically synthesized compounds	Antimicrobial peptides Ribosomal gene-encoded peptides
Examples[1]		 
	Penicillin	Canine cathelicidin Canine β-defensin-1
Molecular characteristics	Small compounds <2000 Da, easy to synthesize with lower production cost	Small amphipathic peptides <10 kDa, feasible to synthesize with higher production cost
Antimicrobial spectrum	Most are active against a subclass of microbes with low side effects	Most are broadly active and multifunctional with potential side effects
Primary mechanism of action	Metabolic inhibition, easy to develop bacterial resistance	Plasma membrane disruption, hard to develop bacterial resistance
Application	Conventional antibiotic therapies	Prebiotics, probiotics; transgenic animals; new generation of antibiotics

[1]Structure information for penicillin is from the public domain (http://www.hopkins-abxguide.org/). Structures of canine β-defensin-1 and cathelicidin were adapted from our previous work (Sang *et al.*, 2005; Sang *et al.*, 2007).

Bacteriocins: bacterial AMPs

Bacteriocins are bacterially produced, small, heat-stable peptides that bacteria use to compete against other bacteria of the same species (narrow spectrum) or against bacteria of other genera (broad spectrum) (Cotter *et al.*, 2005). One or several bacteriocins have been identified or are believed to exist in every species of bacteria and archaea (Cotter *et al.*, 2005; Willey and van der Donk, 2007). A current bacteriocin database (Hammami *et al.*, 2007; http://www.cck.rnu.tn/pfba/bactibase/main.php) lists 145 entries including 39 lanthionine-containing bacteriocins (Class I), 40 non-lanthionine-containing bacteriocins (Class II) and other unclassified entries, likely bacteriolysins (Cotter *et al.*, 2005; Willey and van der Donk, 2007). Class I bacteriocins are small peptides (18–39 residues) and are commonly called lantibiotics because of the lanthionine or β-methyllanthionine residues that they contain. These unusual residues are formed during post-translational modification and enzymatically crosslink a dehydrated serine/threonine to a neighboring cysteine, resulting in intramolecular covalent bridges (Cotter *et al.*, 2005; Willey and van der Donk, 2007). In contrast, Class II bacteriocins constitute a very diverse group and are not subject to this extensive post-translational modification. Class III bacteriolysins are large, heat-labile proteins that catalyze the hydrolysis of bacterial cell walls resulting in autolysis of targeted bacteria. The majority of Class I and Class II bacteriocins are active in the nanomolar range against Gram-positive bacteria in closely related species or in a broad-spectrum manner for many species.

The most promising bacteriocins in development as antibiotics are those produced by lactic acid bacteria (LAB) with the core genera including *Lactobacillus*, *Lactococcus*, *Leuconostoc*, *Pediococcus* and *Streptococcus*. Because of the long history of using LAB in the processing of fermented foods, the antimicrobial and safety information for LAB in food preservation is widely accepted. In addition, LAB are used extensively as probiotics in food processing and preservation (De Vuyst and Leroy, 2007; Sit and Vederas, 2008), and LAB-derived bacteriocins will likely enter the working pharmacopeia as oral or gastrointestinal antibiotics (Rossi *et al.*, 2008). Examples of LAB-derived bacteriocins include nisin, mersacidin, lacticin 481 and lacticin 3147. Among these, nisin has been approved for commercial use in some food processing applications and as an anti-infective for bovine mastitis (Cotter *et al.*, 2005; Dufour *et al.*, 2007), and mersacidin has been evaluated in preclinical tests to treat Gram-positive infections (Hancock and Sahl, 2006). Lacticin 3147, a two-peptide lantibiotic, has shown promise in preventing mastitis infections (Crispie *et al.*, 2005) and as a food preservative (Gardiner *et al.*, 2007). Importantly, several bacteriocins, including lacticin 3147, mersacidin and leucocin A, display potent activity against antibiotic-resistant bacterial strains such as vancomycin-resistant enterococci (VRE) and methicillin-resistant *Staphylococcus aureus* (MRSA) (Kruszewska *et al.*, 2004; Sit and Vederas, 2008). In addition to food-introduced LAB, gastrointestinal commensal LAB (enterococci and streptococci) and microflora of the family Enterobacteriaceae produce a large panel of bacteriocins, such as enterocins, salivaricins, colicins and microcins. These bacteriocins have significant potential for probiotic or antibiotic use after suitable biotechnological modifications are developed (Duquesne *et al.*, 2007; Nes *et al.*, 2007).

Fungal AMPs

The most widely used and historic antibiotic to date, penicillin, is from the fungus *Penicillium chrysogenum*, previously named *Penicillium notatum*. In addition, many peptide antimicrobials are produced by fungi. For example, soil-fungi peptide antibiotics named peptaibols (small peptides usually containing α-aminoisobutyric acid and a C-terminal alcohol) have potent antibacterial and antifungal properties (Duclohier, 2007). The antimicrobial properties of peptaibols derive from their amphipathic, helical structure that facilitates lytic pore formation in membranes. Although peptaibols are peptide antibiotics they are not gene-encoded, ribosomal-produced AMPs; instead they use a multi-enzyme, non-ribosomal peptide sythetase complex (peptaibol synthetase) for their biosynthesis (Leitgeb *et al.*, 2007). Hundreds of peptaibol sequences are compiled in the Peptaibol Database (http://www.cryst.bbk.ac.uk/peptaibol/home.shtml).

Fungal genomes also encode abundant cysteine-rich AMPs consisting of α-helix and β-sheet structures, collectively called defensin-like peptides (Zhu, 2008). Plectasin is the first identified fungal defensin and is active against antibiotic-resistant strains of *Streptococcus pneumoniae* with efficacy in treating peritonitis and pneumonia in mice (Mygind *et al.*, 2005) (Table 1). Bioinformatics analysis of fungal genomes reveals six families of fungal defensin-like peptides, among which three families are ancestral to defensin molecules from plants, insects and invertebrates (Zhu, 2008). Indeed, evolutionarily, cysteine-rich defensin-like peptides have been suggested to be the most diverse group of AMPs existing in all cellular organisms (Yeaman and Yount, 2007; Zhu, 2008).

Plant AMPs

It has been estimated that there are about 300 genes encoding defensins in plants (Silverstein *et al.*, 2005; Thevissen *et al.*, 2007). Although direct antimicrobial activity of most plant defensins against animal pathogens has not been reported, four cysteine-rich plant AMPs and their synthetic analogues are potent against bacterial and fungal infections. These AMPs were isolated from seeds of *Impatiens balsamina*, an herb with a long history of use among Asian people to treat infectious diseases (Thevissen *et al.*, 2007; Marcos *et al.*, 2008). Another fascinating subgroup of plant AMPs are cyclotides characterized by a cyclic backbone and knotted disulfide bonds resulting from cyclization. Plant cyclotides were recently shown to be highly active against HIV-infected cells (Ireland *et al.*, 2008) and to significantly suppress the development of gastrointestinal nematode parasites in livestock (Barbeta *et al.*, 2008; Colgrave *et al.*, 2008).

Animal AMPs

Most animals possess defensins or defensin-like peptides. Indeed defensin-like peptides are present in venom toxins from arthropod and reptile species such as scorpions and snakes (Whittington *et al.*, 2008), and genome analysis of the platypus suggests that defensin-like peptides are an evolutionary signature (Warren *et al.*, 2008; Whittington *et al.*, 2008). Mammals possess the most diverse groups of defensins. Three subgroups of mammalian defensins, α-, β- and θ-defensins, have been classified based on the differential connections of their three disulfide bridges (Ganz, 2003; Selsted and Ouellette, 2005). Defensins have evolved in mammals with α- and θ-defensins only appearing in species later than glires and some primate clades (Selsted and Ouellette, 2005; Lehrer, 2007; Lynn and Bradley, 2007; Sang and Blecha, 2008).

In addition to cysteine-rich AMPs, there are other prominent subgroups of AMPs in animals characterized on the basis of primary or secondary peptide structure (Brogden, 2005). One subgroup is characterized by an abundant number of cationic peptides containing basic and/or hydrophobic residues at a high ratio and many of them conform into α-helical structures in membrane-mimetic environments. Examples of these subgroups include mammalian cathelicidins, amphibian magainins and maximins, and insect cecropins. Some members of this subgroup, such as cathelicidins, are rich in certain residues such as proline (e.g. porcine PR-39), phenylalanine (e.g. porcine prophenins) and tryptophan (e.g. bovine indolicidin), which may contribute to their 'multi-hitting' model of antimicrobial responses (McPhee and Hancock, 2005; Hancock and Sahl, 2006; Hale and Hancock, 2007) (Fig. 1). The second subgroup of AMPs includes antimicrobial fragments derived from large proteins such as lactoferricin from lactoferrin and the antimicrobial domain of lysozyme (Brogden, 2005) (Table 1). Potent activity against a broad spectrum of micro-organisms including bacteria, fungi, enveloped viruses and tumor cells has been observed in members of these animal AMPs (Brogden, 2005) plus multiple roles with respect to immunoregulation and cell signaling (Hancock and Sahl, 2006; Zaiou, 2007). These examples illustrate the diversity of AMPs and suggest that they may be a rich source for future antibiotic design and drug development. Indeed, multiple AMPs or analogues are in development for antimicrobial and immunoregulatory therapies (Gordon *et al.*, 2005; McPhee and Hancock, 2005; Hancock and Sahl, 2006).

AMPs as natural antibiotics: antimicrobial activity, mechanism of action and current status in development

Similar to traditional antibiotics, most AMPs are highly active against bacteria and fungi. However, many AMPs,

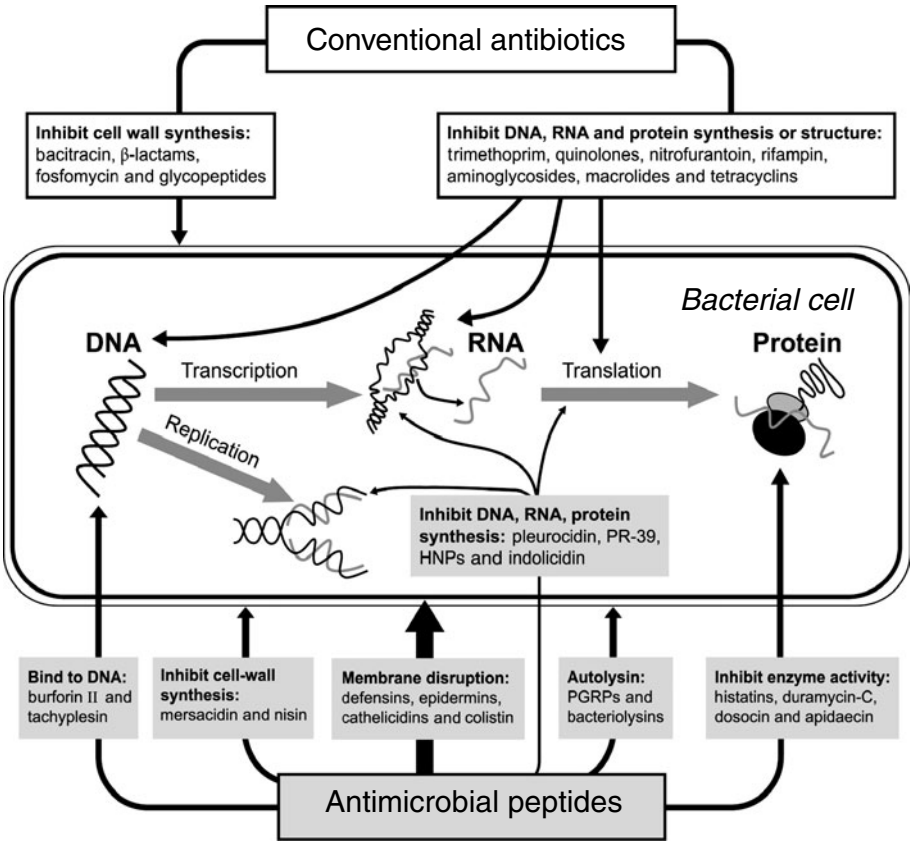


Fig. 1. Mechanisms of action of conventional antibiotics and AMPs. Bactericidal mechanisms of AMPs are illustrated with an emphasis on the propensity to membrane disruption. AMP-related aspects were adapted with permission from Macmillan Publishers: *Nature Reviews: Microbiology* (Brogden, 2005) and *Nature Biotechnology* (Hancock and Sahl, 2006). Conventional antibiotic information was adapted from a public domain (http://www.hopkins-abxguide.org/).

including plant cyclotides, and animal defensins and cathelicidins, also possess antiviral or anti-parasite activity (Table 1). Generally, bacteriocins are extremely active against related Gram-positive bacteria or Enterobacteriaceae with minimal inhibitory concentration (MIC) in the nanomolar range. Several bacteria that produce bacteriocins are food-borne pathogens, such as *Listeria monocytogenesis* and antibiotic-resistant strains of *Salmonella* spp., *Campylobacter* spp., *Escherichia coli* and *Enterococcus* spp. (Kang and Lee, 2005; Nes *et al.*, 2007; Sit and Vederas, 2008; Svetoch *et al.*, 2008). Therefore, bacteriocins, especially lantibiotics, have promise as antibiotics to meet the requirements of food preservation and in preventing infections from food-borne pathogens (Nes *et al.*, 2007; Sit and Vederas, 2008). The primary bactericidal mechanism of bacteriocins is through membrane pore formation as shown for most lantibiotics. However, some bacteriocins also attack other cellular targets such as mersacidin interaction with lipid II to prevent cell-wall synthesis, microcin J25 inhibition of bacterial RNA synthesis and duramycin-C inhibition of bacterial phospholipase A2. Other bacteriocins like nisin use more than one mechanism (membrane pore formation and disruption of cell-wall synthesis) to kill targeted bacteria. The antimicrobial capacity of two-peptide bacteriocins such as Class I lacticin 3147 and Class II lactacin-F requires the combined activity of both partners to dissipate membrane potential, to induce ion leakage and/or to interfere with cellular ATP production (Fig. 1) (Cotter *et al.*, 2005; Willey *et al.*, 2007).

Membrane-targeting mechanisms are the most conserved killing mechanisms of AMPs identified from plants, insects and vertebrate animals (Table 1 and Fig. 1). Through their net-positive surface charge and/or amphipathic structure, AMPs undergo a pore-formation process of membrane attachment, insertion and permeabilization (Brogden, 2005). In a recently proposed two-state model based on studies of representative cysteine-rich AMPs, it is posited that AMPs initially assemble parallel to the plane of the membrane and cause membrane thinning in proportion to the peptide/lipid ratio of the membrane. As the peptides continue assembling on the membrane surface and exceed the peptide/lipid ratio threshold, the interaction enters a second state, where alignment of the peptide assemblages becomes perpendicular to the plane of the membrane and form transmembrane pores (Huang, 2006; Jang *et al.*, 2006). Using solid-state NMR techniques, Mani *et al.* (2006) accurately measured the properties of

the lytic pores induced by porcine protegrin (a cysteine-rich cathelicidin) on a lipid bilayer that mimics the bacterial membrane. Although AMPs with diverse structural properties may undergo different kinetic processes, this model shows that cysteine-rich AMPs like defensins and protegrins insert a pore in the lipid bilayer through a β-barrel mechanism. In addition, due to the distinct lipid composition (mainly cholesterol content) and relative neutral charge of eukaryotic membranes compared with bacterial membranes, the effective concentration of AMPs to induce changes in eukaryotic membranes is higher than the lytic dose on bacteria (Huang, 2006; Jang *et al.*, 2006; Mani *et al.*, 2006). The different kinetics of AMP interaction with eukaryotic and bacterial membranes partially explain why host AMPs discriminate between host cells and bacteria. In addition, many higher vertebrates have evolved other mechanisms to target their AMPs to pathogens while limiting damage to themselves, including (i) storage of potent AMPs in special cells or cell compartments such as neutrophils and neutrophil granules (Selsted and Ouellette, 2005; Lehrer, 2007), (ii) controlled synthesis of mature AMPs only in functional organs such as synthesis of killing toxins in venom glands of arthropods, reptiles and the platypus (Warren *et al.*, 2008), (iii) differential processing of AMP's mature peptides to balance antimicrobial activity such as multiple mature forms of human LL-37 in sweat on the skin surface (Murakami *et al.*, 2004), and (iv) interference with AMP activity by other coexisting molecules such as serum proteins and mucins (Brogden, 2005). Similarly, bacteria also have protective mechanisms to limit harm from self-produced bacteriocins. For example, the genes for lantibiotic biosynthesis, regulation and self-immunity are found in clusters allowing for coordinated expression (Rossi *et al.*, 2008). Furthermore, in the case of lantibiotics, immunity is provided by specific immune proteins and/or by sensing proteins to regulate bacteriocin synthesis or transport. For example, a single immune protein PepI protects *Staphylococcus epidermidis* from its bacteriocin Pep5 by masking the target molecule of Pep5 on the membrane. NisI, which is an outer membrane lipoprotein of *Lactococcus lactis*, arrests nisins to limit local concentrations from reaching the membrane of nisin-producers; however, NisI cannot protect *L. lactis* from closely related lantibiotics produced by *Bacillus subtilis*, indicating that self-immunity to a bacteriocin is very specific (Willey *et al.*, 2007; Draper *et al.*, 2008).

The overall antimicrobial effect of an AMP *in vivo*, which is manifested by suppression/elimination of infection by a pathogen, can result from both its direct antimicrobial activity and indirect immune regulatory functions. In this context, most AMPs in higher vertebrates, such as mammalian defensins and cathelicidins, have been shown to be multifunctional and because of this property are often referred to as host defense peptides (Hancock and Sahl, 2006; Zaiou, 2007). A partial list of these immunoregulatory functions exerted by mammalian antimicrobial host defense peptides includes chemoattractant activity for immune cells, inhibition of oxidative burst of phagocytes, promotion of angiogenesis and wound healing, regulation of development and function of male reproductive cells, and induction of autoimmunity (Hancock and Sahl, 2006; Zaiou, 2007). Although these multifunctional properties may increase the drug development potential of AMPs, some may also cause limitations in the development of antibiotics. Other challenges to AMP-based drug development include cytotoxicity and the higher cost of peptide synthesis (Hancock and Sahl, 2006; Scott *et al.*, 2007). Finally, although microbial resistance is usually considered less likely for AMPs than conventional antibiotics, some mechanisms of resistance to AMPs have been identified (Gunn, 2008; Kraus and Peschel, 2008); this should be considered in developing and using AMP-based drugs.

Several recent publications have discussed and reviewed AMP-based drug development (Andrès and Dimarcq, 2005; Gordon *et al.*, 2005; McPhee and Hancock, 2005; Hancock and Sahl, 2006). Most AMP-based antibiotic studies are in the discovery or preclinical stages with some proceeding to clinical trials. Nisin, a LAB lantibiotic, is one of few examples of AMP-based antibiotic therapies that have been commercialized. Other AMP-based drugs that have progressed to clinical trials, such as those derived from insect cecropin B and bovine indolicidin (Hancock and Sahl, 2006; Scott *et al.*, 2007), have been developed to treat wounds or skin-related infections in humans, applications that may also be used in veterinary medicine. Some drugs in testing are derivatives of AMPs that have been modified to improve their antimicrobial activity. These modifications include introducing non-natural residues like D-amino acids, addition of C-terminal amidation and catalysis of cyclic formation, which are believed to improve stability and activity against targeted micro-organisms as shown in natural bacteriocins, plant cyclotides and primate θ-defensins (Lehrer, 2007; Bansal *et al.*, 2008; Ireland *et al.*, 2008). Therefore, optimized design of synthetic peptides based on knowledge from natural AMP studies (the concept of 'designer AMPs') may provide a feasible way to increase novel drug development (Scott *et al.*, 2007; Jenssen *et al.*, 2008).

Generation of transgenic animals and plants by xenobiotic expression of an AMP from other species is another approach to improve disease resistance and growth performance in food animals. Transgenic animals are also potent bioreactors to produce AMP-containing prebiotics or to purify natural AMPs. For example, transgenic cows (Hyvönen *et al.*, 2006), rabbits (Han *et al.*, 2008) and goats (Zhang *et al.*, 2008) expressing human lactoferrin in milk were produced to enhance health effects for dairy consumers and to provide large-scale production of human lactoferrin. Although transgenic cows with xenobiotic expression of human

lactoferrin did not exhibit enhanced protection against an *E. coli* intramammary infection (Hyvönen *et al.*, 2006), transgenic mice expressing porcine lactoferrin in milk promoted offspring growth (Wu *et al.*, 2007) and resistance to foot-and-mouth disease (Chen *et al.*, 2008). Broiler diets containing rice that was genetically altered to express human lactoferrin or lysozyme protected chick intestinal tracts similar to subtherapeutic antibiotics and improved small intestinal architecture (Humphrey *et al.*, 2002). Moreover, transgenic mice with ectopic expression of human intestinal defensin-5 (HD-5) (Salzman *et al.*, 2003), porcine cathelicidin PR-39 (Lee *et al.*, 2005) and protegrin-1 (Cheung *et al.*, 2008) had significantly enhanced protection against enteric salmonellosis, bacterial skin infection and *Actinobacillus suis* infection, respectively. In contrast, transgenic mice overexpressing mouse AMPs, such as β-defensin-6 (Yamaguchi *et al.*, 2007) or mouse cathelicidin (Lee *et al.*, 2005), exhibited no increased resistance.

Concluding remarks

AMPs, a group of innate immune effectors with special antimicrobial mechanisms that have endured the selective pressure of years of evolution, provide an attractive platform from which to develop novel antibiotics (Beutler, 2004). The recent realization that AMPs are an essential component of microbe–host mutualism underscores the important immunoregulatory role of AMPs in addition to their well-known direct antimicrobial activity. Thus, these peptides will continue to be investigated for novel therapeutic strategies based on their multifunctional properties as antimicrobials and host defense peptides (Hancock and Sahl, 2006; Hoskin and Ramamoorthy, 2008; Steinstraesser *et al.*, 2008).

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