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Antimicrobial Peptides: Diversity and Perspectives for Their Biomedical Application

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1. Introduction

For over fifty years, people have used antibiotics to treat illnesses caused by pathogens. However, the excessive and inappropriate use of these antibiotics in clinical treatment of humans and animals has increased pathogen resistance to these compounds, turning them into less effective agents. There has also been an increase in the generation of multidrug-resistant pathogens, primarily bacteria and fungi that resist the effects of most currently available antibiotics (Heuer et al., 2006; Field, 2010).

Until now, the pharmaceutical industry is facing this problem by looking for new antibiotics or modifying existing ones. However, pathogens have proven to have the ability to quickly develop and disseminate resistance mechanisms, which compromises this strategy, becoming it less effective. This clearly shows the need to develop new biomedical treatments with different action mechanisms from those of conventional antibiotics (Parisien et al., 2008).

This problem has led that efforts being made on research and development of new biomedical alternatives, among which antimicrobial peptides (AMPs) are considered one of the most promising options. AMPs are produced by a wide variety of organisms as part of their first line of defense (eukaryotes) or as a competition strategy for nutrients and space (prokaryotes). These molecules are usually short peptides (12-100 amino acid residues); have a positive charge (+2 to +9), although there are also neutral and negatively charged. They are amphipathic and have been isolated from bacteria, plants and animals, including humans; which give us an overview of the enormous structural diversity of these molecules and their different action mechanisms (Murray & Liu, 2008).

The continuous discovery of new AMPs groups in diverse organisms has turned these natural antibiotics into the basic elements of a new generation of potential biomedical treatments against infectious diseases in humans and animals. Besides the above, the broad spectrum of biological activities reported for these molecules suggests a potential benefit in cancer treatment, viral and parasitic infections and in the modulation of the immune system, which reinforces the importance of studying these molecules (Mercado et al., 2005; Schweizer, 2009).

The contents of this chapter shows the importance of AMPs for living organisms, not only from the antimicrobial point of view, but also in bacterial cell communication processes, immune response modulation in animals and plant defense mechanisms. It also emphasizes on AMPs' biological and structural diversity, as well as their various action mechanisms and, finally, their possible biotechnological development for the pharmaceutical industry is discussed.

2. AMPs from Gram positive bacteria and their classification

During their evolution, bacteria have acquired mechanisms that allow them to have success in competition for nutrients and space in their habitat. These mechanisms include from the enhancement of chemotaxis systems to the acquisition of defense systems such as the production of antimicrobial peptides (AMPs), also called bacteriocins (Riley & Wertz, 2002). AMPs are biologically active molecules that have the ability to inhibit the growth of other members of the same specie or members of different bacterial genres (Cotter et al., 2005b).

These molecules are synthesized by the vast majority of bacterial groups; in fact, it has been proposed that 99% of bacteria produce at least one, as they have been found in most examined species, covering Gram positive and Gram negative bacteria and archaea; in addition they are used as an important tool in evolutionary and ecological studies (Klaenhammer, 1988). Also, the successful commercial development of nisin (produced by *Lactococcus lactis*) and the use of molecular biology and genetic engineering tools in recent years have provoked a resurgence in AMPs studies, particularly in relation to their potential biomedical applications (Cotter et al., 2005a, b; Bierbaum & Sahl, 2009; Field et al., 2010).

AMPs from Gram positive bacteria represent a heterogeneous group of chemical molecules; nevertheless only three main categories have been established based on their structural modifications, size, thermostability and action mechanisms (Table 1). Class I (lantibiotics) is constituted by cationic peptides ranging from 19 to 38 amino acid residues, which undergo posttranslational modifications and exert their effect at membrane and cell wall levels. Their posttranslational modifications are diverse; the most important involve dehydration reactions of serine and threonine residues, resulting in the formation of didehydroalanine (Dha) and didehydroaminobutyric acid (Dhb), respectively (Cotter et al., 2005b). The reaction of these amino acids with the thiol group (SH) of a cysteine residue generates a thioether bond producing lanthionine (in the case of Dha) and β-methyl-lanthionine (in the case of Dhb). The formation of these bonds within the peptide generates a series of "globular" structures that are characteristic of lantibiotics. This AMPs class is further divided into subgroups A and B, having nisin as the representative member of subgroup A, while mersacidin, produced by bacteria of the *Bacillus* genus, is a member of subgroup B (Table 1) (McAuliffe et al., 2001; Cotter et al., 2005a).

On the other hand, class II (non lantibiotics) is formed by AMPs constituted by 30 to 60 amino acid residues; they do not contain lanthionine, are thermostable and induce the formation of pores in the membrane of target cells. These peptides in turn are divided into subclasses IIa, IIb, IIc and IId (Table 1). Subclass IIa is the largest and its members posses the amino terminal motif YGNGVXCXXXXVXV (X indicates any amino acid residue) and have one or two disulfide bonds. AMPs from this subclass show specific activity against the bacteria *Listeria monocytogenes* (Ennahar et al., 2000). Leucocin A from *Leuconostoc gelidum* is a representative member of this subclass (Hastings et al., 1991).

Class	Subclass	Representative AMPs	Producing bacteria
I Lantibiotic	I A	Nisin	*Lactococcus lactis*
I Lantibiotic	I B	Mersacidin	*Bacillus* spp.
II Non lantibiotic	IIa	Leucocin A	*Leuconostoc gelidum*
II Non lantibiotic	IIb	Lactococcin G	*L. lactis*
II Non lantibiotic	IIc	AS-48 enterocin	*Enterococcus faecalis*
II Non lantibiotic	IId	Lactococcin A	*L. lactis*
III Proteins		Helveticin J	*L. helveticus*

Table 1. Classification of AMPs found in Gram positive bacteria (Cotter et al., 2005a; Drider et al., 2006)

Subclass IIb comprises AMPs that require the combined action of two peptides in order to have activity; these peptides do not show inhibitory activity on an individual basis. Lactococcin G from *L. lactis* is a representative member of this subclass (Moll et al., 1996). The AMPs that make up subclass IIc posses a cyclic structure as a result of the covalent binding of their carboxyl and amino terminal ends; AS-48 enterocin from *Enterococcus faecalis* is one of the main representatives of this subclass (Sánchez et al., 2003). Subclass IId is formed by a variable group of linear peptides, among which lactococcin A from *L. lactis* is found (Holo et al., 1993). Finally, the class III is formed by proteins with molecular masses higher than 30 kDa, the helveticin J from *L. helveticus*, is an example (Drider et al., 2006).

2.1 Genes involved in AMPs synthesis and expression regulation from Gram positive bacteria

The genes encoding AMPs are organized as operons, which could contain several genes involved in the synthesis and regulation. For example, the enterocin A operon of *Enterococcus faecium* contains the *entA* gene that codifies for pre-enterocin; in addition, this operon contains the genes that codify for the protein involved in the self-protection of the producing strain (*entI*), the AMP synthesis induction gene (*entF*), genes for proteins involved in extracellular transport (*entT, D*), as well as the genes of proteins related to the AMP synthesis regulation (*entK, R*) (Nilsen et al., 1998). In the case of lantibiotics, these have additional genes that codify for AMP modification enzymes (McAuliffe et al., 2001).

AMPs synthesis regulation is mediated by two signal transduction systems constituted by two or three components. Diverse factors activate these systems, which include: the presence of other competing bacteria (Maldonado et al., 2004), temperature or pH stress (Ennahar et al., 2000) and a mechanism of "quorum sensing" (Kuipers et al., 1998). An

interesting example is the three-component system that regulates the synthesis of enterocin A in *E. faecium*, which is regulated by the mechanism of quorum sensing. This system includes: 1) a histidine kinase (HK), located in the cytoplasmic membrane which detects extracellular signals, and 2) a cytoplasmic response regulator (RR) that mediates an adaptive response, which usually is a change in the gene expression and an induction factor (IF), whose presence is detected by the HK protein (Figure 1, stage 1) (Cotter et al., 2005b). In this case, the system is triggered as a result of an IF excess concentration through a slow accumulation during cell growth, the HK detects this concentration and initiates the signaling cascade that activates the transcription of genes involved in enterocin A synthesis (Figure 1, stages 2 and 3) (Ennahar et al., 2000). Other examples of this type of regulation include several class II members such as sakacin P and A from *Lactobacillus sake* (Hühne et al., 1996). Moreover, some examples of regulation mediated by two-component systems include numerous lantibiotics, for example, subtilin from *Bacillus subtilis* and nisin from *L. lactis*. In these systems AMPs have a dual function, as they have antimicrobial activity and also act as a signal molecule by inducing its own synthesis (not shown) (Kleerebezem, 2004).

2.2 AMPs secretion and self-protection mechanisms from Gram positive bacteria

AMPs are synthesized as inactive pre-peptides containing a signal peptide at the N-terminal region (Figure 1, stage 3). This signal keeps the molecule in an inactive form within the producing cell facilitating its interaction with the carrier, and in the case of lantibiotics plays an important role in the pre-peptide recognition by the enzymes that perform posttranslational modifications. The signal peptide may be proteolytically removed during transport of the pre-peptide into the periplasmic space by the same transport proteins (ATP-dependent ABC membrane transporters, which may also contain a proteolytic domain) (Figure 1, stage 4), or by serine-proteases present on the outside of the cell membrane. Thus, the carboxyl terminus is separated from the signal peptide and is released into the extracellular space to produce the biologically active peptide (Figure 1, stage 5) (Ennahar et al., 2000; Cotter et al., 2005b).

AMPs producing bacteria possess proteins that protect them from the action of their own peptides. The exact molecular mechanisms by which these proteins confer protection to the producing bacteria are unknown; however, two protection systems have been proposed, which, in some cases act in the same bacteria (Kleerebezem, 2004). The protection can be provided by a specific protein that sequesters and inactivates the AMP, or that binds to the AMP receptor causing a conformational change in its structure making it inaccessible to the AMP (Figure 1, stage 6) (Venema et al., 1994). The second system is constituted by the ABC transport proteins, which in some cases provide the protection mechanism through the expulsion of the membrane-binding AMPs (Otto et al., 1998).

2.3 AMPs spectrum and action mechanisms from Gram positive bacteria

In general, the antibacterial action spectrum of AMPs of Gram positive bacteria is restricted to this bacterial group. However, there are several molecules with a wide range of action, inhibiting the growth of Gram positive (McAuliffe et al., 2001) and Gram negative bacteria (Motta et al., 2000), human pathogenic fungi (De Lucca & Walsh, 1999) and viruses (Jenssen et al., 2006). Also, AMPs have activity against various eukaryotic cells, such as human and bovine erythrocytes (Datta et al., 2005). With regard to their antimicrobial activity, AMPs possess essential characteristics in order to carry out the activity, regardless of their target

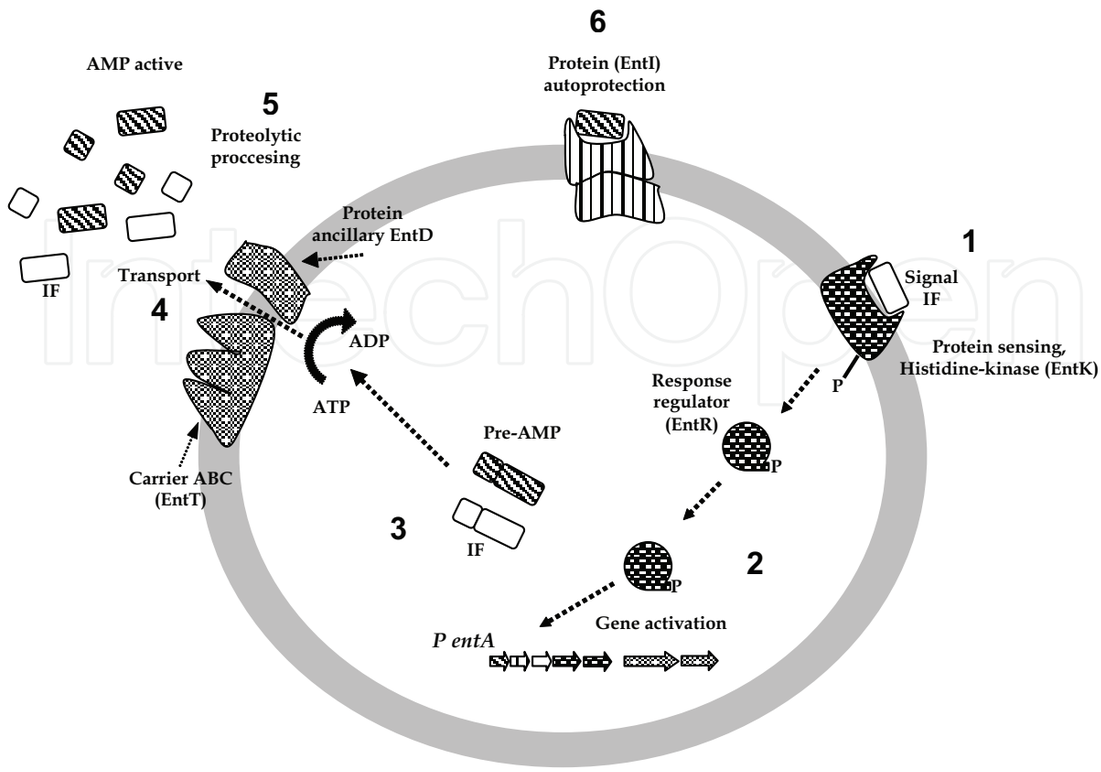

Fig. 1. Regulation of the synthesis of enterocin A from *Enterococcus faecium* (non-lantibiotic). Stage 1, the EntK protein detects the presence of the induction factor (IF) and autophosphorylates. Stages 2 and 3, the phosphate group is transferred to the EntR response regulator, which activates genes involved in the synthesis of the pre-peptide (pre-enterocin A) and of the IF. Stages 4 and 5, the pre-enterocin A and the IF are transported to the outside by the EntT and EntD proteins, and processed by the same system, releasing the active enterocin A and the IF. Stage 6, the EntI protein protects the producing bacteria from the effect of enterocin A (Ennahar et al., 2000; Cotter et al., 2005b)

cell. These include, 1) a net positive charge which favors its interaction with the negatively charged lipopolysaccharide membrane of Gram negative bacteria, or with teichoic and lipoteichoic acids from the wall of Gram positive bacteria; 2) hydrophobicity, required for the insertion of the AMP in the cell membrane; and 3) flexibility, which allows a conformational change from a soluble state to one of membrane interaction. These characteristics vary from molecule to molecule; however, all are important for antimicrobial activity (Jenssen et al., 2006).

It has been shown that the action targets of AMPs studied to date are the cell membrane and wall, as well as some important enzymes for cell metabolism. The action mechanisms include: *i*) pore formation in the cell membrane, causing loss of cell contents, this is the mechanism described for nisin (Enserink, 1999) and lactococcin A from *L. lactis* (Van Belkum et al., 1991); *ii*) cell wall synthesis inhibition, this mechanism has been described for mersacidin, which involves binding to lipid II, the main transporter of peptidoglycan subunits (UDP-Mur -Nac-pentapeptide-GlcNAc) (Brotz et al., 1995); and *iii*) inhibition of the activity of enzymes such as phospholipase A2, which is involved in membrane repair; this is the reported mechanism for cinamicin from *Streptomyces cinnamoneus* (Marki et al., 1991).

Additionally, there have been reports of AMPs that possess a dual action mechanism, such as nisin (Figure 2) (Breukink et al., 1999; Bierbaum & Sahl, 2009). The most accepted model showing the dual action mechanism of nisin proposes that it initially binds to the cell wall by electrostatic attraction, events that are facilitated due to the positive charge of this peptide and negative charges of cell wall components (Figure 2, stage 1). Subsequently, nisin binds to lipid II, the main transporter of peptidoglycan subunits, and uses this molecule to anchor itself to the cell membrane (Figure 2, stage 2). Then, it changes its orientation with respect to the membrane and inserts itself in it; this involves the translocation of its carboxyl terminus through the membrane. Finally, the binding of different peptides in the insertion site leads to the formation of a transmembrane pore that allows the exit of important molecules such as amino acids and ATP, leading the bacteria to a rapid cell death (Figure 2, stage 3) (Wiedemann et al., 2001; Bierbaum & Sahl, 2009).

2.4 AMPs resistance from Gram positive bacteria

Resistance development in pathogenic bacteria that are normally sensitive to AMPs is of great interest because of their possible use in biomedical therapies, as bacterial resistance might limit their use. Within a particular bacterial species there may be naturally resistant members to AMPs or resistance may arise as a result of continuous exposure; which are known as intrinsic and acquired resistance, respectively (Xue et al., 2005).

Most research in this area has focused on specific AMPs such as nisin and class IIa members. In the first case, *L. monocytogenes*, *L. innocua*, *Streptococcus pneumoniae* and *S. bovis* resistant mutants have been detected, whose resistance has been correlated to changes in the wall and cell membrane (Gravesen et al., 2002). More specifically the synthesis and incorporation of various structural components to the membrane (Li et al., 2002) and the cell wall (Mantovani & Russell, 2001) have been observed in the mutants, which has favored an increase in positive charges in these cell structures and reduced the antibacterial activity of nisin (which has a net positive charge). Likewise, changes in the fluidity of cell membrane (Verheul et al., 1997) and an increase in the thickness of the cell wall of some mutant bacteria have been observed (Maisnier & Richard, 1996; Murray & Liu, 2008).

The mechanisms of resistance to type II AMPs have been studied in strains of *L. monocytogenes*, essentially towards class IIa peptides, in which the resistance is related to several factors including reduced expression of a permease that acts as a potential receptor (Dalet et al., 2001), as well as changes in membrane fluidity (Vadyvaloo et al., 2002), and in cell surface charges (Vadyvaloo et al., 2004). The importance of studying the resistance lies not only in the possible long term ineffectiveness of AMPs, but also in generating knowledge that could serve as a basis for strategies to improve the therapeutic potential of these antimicrobial molecules, i.e. the development of protein engineering strategies to improve the biological properties of AMPs (Field et al., 2010).

Currently, the existence of natural AMPs variants suggests that there is flexibility in the location of some important amino acid residues for antimicrobial activity, which indicates that it is possible to generate mutants with changes that increase this activity. Thus, additional studies are needed to determine the mechanisms of resistance to AMPs, as well as the frequency with which it occurs (Cotter et al., 2005a).

2.5 Current and potential Gram positive AMPs applications in biomedical therapies

AMPs null toxicity to humans and animals and activity directed towards pathogenic bacteria has allowed investigating their potential applications in biomedical therapies. In particular, the action mechanisms of these peptides and their activity against pathogens

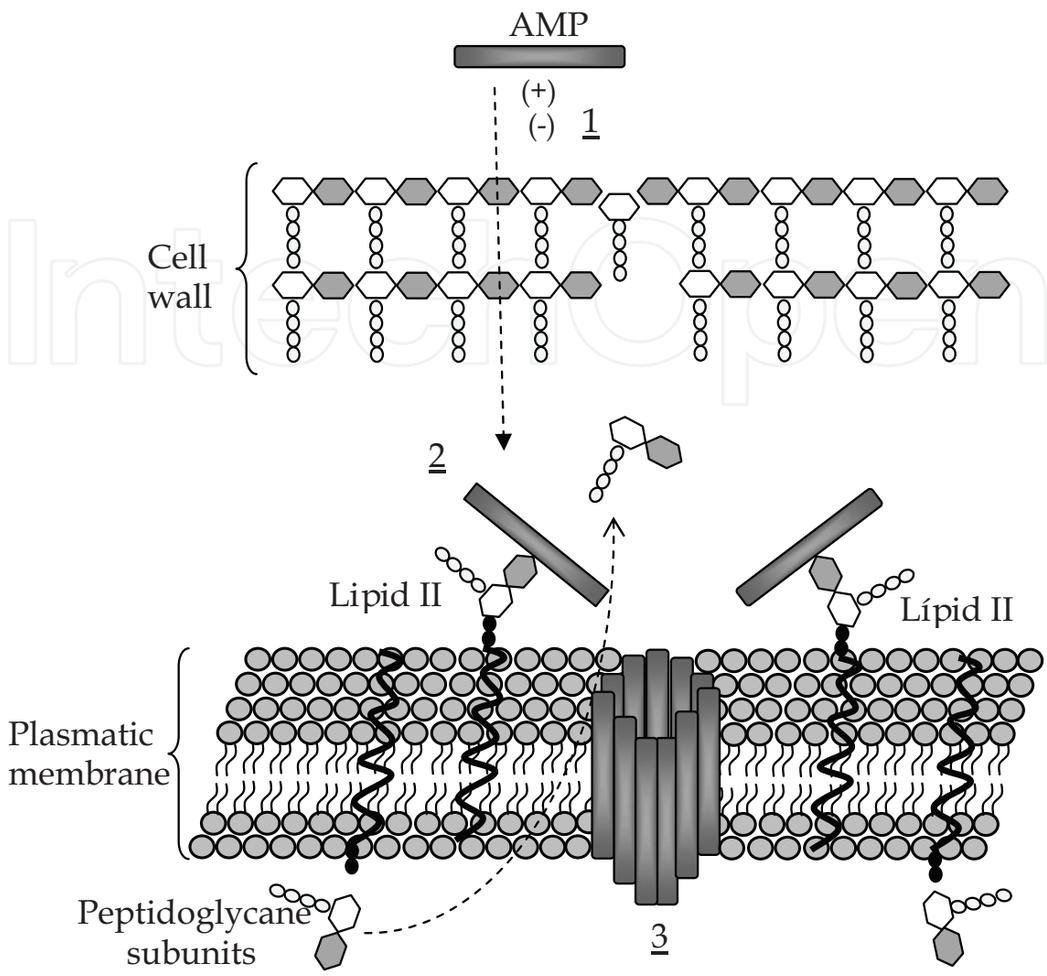

Fig. 2. Model showing the dual action mechanism of nisin from *Lactococcus lactis*. Stage 1, nisin has a net positive charge that increases its interaction with the negative charges of the cell wall components. Stage 2, nisin binds to lipid II, the main transporter of peptidoglycan subunits from the cytoplasm to the cell wall, interfering with its synthesis, leading the bacteria to cell death. Stage 3, in addition, several nisin molecules use lipid II to anchor and insert themselves into the cell membrane and begin the formation of pores, leading the bacteria to a rapid cell death (Wiedemann et al., 2001; Cotter et al., 2005a)

resistant to conventional antibiotic therapy, making them an attractive option as antimicrobial agents (Table 2) (Cotter et al., 2005a, b; Piper et al., 2009). Broad spectrum AMPs or bioengineered AMPs could be used against Gram positive pathogens of humans and animals. For example, lacticin 3147 from *L. lactis* has shown *in vitro* activity against methicillin-resistant *Staphylococcus aureus* (MRSA); vancomycin-resistant enterococci (VRE); vancomycin-intermediate *S. aureus* (VISA); streptococci, *S. pneumoniae, S. pyogenes, S. agalactiae, S. dysgalactiae, S. uberis, S. mutans*; *Clostridium botulinum* and *Propionibacterium acnes* (Galvin et al., 1999; Piper et al., 2009). In the same way, it has been created two nisin variants by bioengineered (nisin V and nisin T) with enhanced antimicrobial activity against Gram positive pathogens like MRSA, VRE, VISA, *Clostridium difficile*, *L. monocytogenes* and *B. cereus* (Field et al., 2010).

AMPs and producing strain	Activity	Potential biomedical applications
Nisin *L. lactis*	Inhibits Gram positive and Gram negative bacteria, including *Helicobacter pylori*	Bacterial mastitis, oral hygiene, treatment of methicillin-resistant *Staphylococcus*, enterococcal infections, topical formulations, deodorants and cosmetics, treatment of peptic ulcers and enterocolitis
Epidermin *S. epidermidis*	Inhibits *Propionibacterium acnes*, staphylococci, streptococci	Acne, folliculitis, impetigo
Mersacidin *Bacillus* spp.	Inhibits staphylococci and streptococci strains	Treatment of methicillin-resistant *Staphylococcus aureus* and streptococcal infections
Cinamicin *Streptomyces cinnamoneus*	Phospholipase A2 inhibitor, angiotensin and HSV converting enzyme	Inflammation reduction, blood pressure regulation and viral infection treatment

Table 2. A few Gram positive AMPs examples and their potential biomedical use (Cotter et al., 2005a)

On the other hand, *in vivo* experiments using animal models have shown positive results after using lantibiotics, such as mersacidin and nisin in the treatment of respiratory tract infections caused by *S. aureus* MRSA (Kruszewska et al., 2004; De Kwaadsteniet et al., 2009), and *Streptococcus pneumonia* (Goldstein et al., 1998), in addition to skin care and oral therapies, such as tooth paste for prevention of teeth loss, bad breath and gingivitis (Howell et al., 1993; Arauz et al., 2009). Likewise, nisin has showed that has the potential for treatment of human mastitis (Fernández et al., 2008).

The Oragenics pharmaceutical company has realized extensive preclinical testing on the lantibiotic mutacin MU1140 of *S. mutans,* which has demonstrated activity against wide variety of disease-causing Gram positive bacteria, including MRSA, VRE, *Mycobacterium tuberculosis*, and anthrax. For the complete trials, this company has created the synthetic version MU1140-S, and they expect to conclude the preclinical testing in 2011. Likewise, in New Zealand, the BLIS K12® dietary supplement is sold as an inhibitor of bacteria responsible for bad breath, because it contains a strain of *S. salivarus* that produces salivaricin A2 and B peptides (Tagg, 2004).

In relation to animal disease, several AMPs have been proposed as potential alternatives to bovine mastitis control. Nisin has activity against mastitis pathogens and has been formulated in Wipe Out® and Mast Out®, commercially available products (Ryan et al., 1998; Wu et al., 2007). Also, AMPs produced by *S. aureus*, *S. epidermidis* and *Streptococcus gallolyticus* have been tested against strains of both *S. aureus* and *Streptococcus* species

isolated from bovine mastitis (Varella et al., 2007; Pieterse et al., 2008). Finally, *B. thuringiensis* AMPs have showed inhibitory action against *S. aureus* isolates from bovine mastitis (Barboza-Corona et al., 2009).

From a non antimicrobial medical perspective, AMPs such as cinamicin may have different biomedical applications, because this peptide inhibits the function of phospholipase A2 and the angiotensin converting enzyme, which are involved in the immune system and in maintaining blood pressure in humans, respectively; so that they could be used in inflammatory processes and in blood pressure regulation (Ennahar et al., 2000) (Table 2). In the same way, nisin has shown contraceptive activity (Gupta et al., 2009) and protector activity in rabbits and mice vaginas in *in vitro* and *in vivo* studies (Reddy et al., 2004).

3. AMPs from Gram negative bacteria and their classification

The term "bacteriocinogenicity" is used to describe the ability of Gram negative bacteria to synthesize and excrete AMPs (Daeschel et al., 1990). These molecules were first detected in *Escherichia coli* and were called colicins. Later, they were found in Gram positive bacteria and have been studied with great interest, especially those produced by lactic acid bacteria, which can be used in food preservation because its activity against Gram negative bacteria, the leading cause of food poisoning (Hardy, 1975; Tagg et al., 1976). Colicin V from *E. coli* and pyocin from *Pseudomonas aeruginosa*, are the two best studied peptides in the Gram negative bacteria group (Table 3) (Jack et al., 1995).

The colicin group has been taken as the representative group of Gram negative AMPs, although there are differences between them. Pyocins are AMPs of high molecular weight synthesized by *P. aeruginosa* strains, which could participate in establishing and protection of bacteria. There are three types of pyocins: R, F and S, which resemble the tails of bacteriophages of the Myoviridae family. Type R pyocins show broad similarities with the fibers of the tails of these phages. Type R pyocins are contractile and not flexible, the F type are flexible, but are not contractile; and the S type are susceptible to proteases (Michel-Briand & Baysse, 2002; Waite & Curtis, 2009).

The colicins are proteins between 29 and 90 kDa, which have binding, transport and specific activity domains, same as those found in pyocins. The secretion of colicins is carried out in cell lysis, which involves their death (Riley & Wertz, 2002; Sano et al., 1993). Other kind of AMPs produced by *E. coli* and other enterobacteria are the microcins, which are a group of circular peptides, from which microcin J25, produced by *E. coli* AY25, has been taken as a model (Craik et al., 2003). Microcins are low molecular weight molecules under 10 kDa, which play an important role in competition for colonization of the gastrointestinal tract. They are generally hydrophobic, highly stable in relation to heat, extreme pH and proteases (Duquesne et al., 2007). Some other Gram negative AMPs are: Serracin P, produced by *Serratia plymithicum* J7; mundticin KS, synthesized by *Enterococcus mundtii*, NFRI 7393 and caratovoricin, produced by *Pectobacterium carotovorum* subsp. *carotovorum* (Jabrane et al., 2002; Kawamoto et al., 2002; Yamada et al., 2008).

3.1 Genes involved in Gram negative AMPs synthesis

The genes required for colicin synthesis are encoded usually in plasmids, and consist of a colicin gene, a gene for immunity and a lysis gene. Most of the genes coding for AMPs in Gram negative bacteria probably derived from recombination of existing AMPs genes. Colicins contains a central domain (50%) involved in the recognition of the target cell receptor; a N-terminal domain (> 25%) responsible for the translocation of the peptide to the

AMPs and producing bacteria	Group	Main features
Colicin *Escherichia coli*	Group A	N-terminal domain rich in glycine (~20-40%)
	Group B	N-terminal domain rich in glycine (~10-20%)
Microcins *E. coli*	Class I	The self-immunity genes are not close to microcin structural gene
	Class IIa	Cluster of four genes encoded in plasmids
	Class IIb	Chromosomally encoded, have a complex transcriptional organization
Pyocins *P. aeruginosa*	Type R	Resemble the fibers of the tails of bacteriophages of the Myoviridae family and are contractile but are not flexible
	Type F	Flexible, but are not contractile peptides
	Type S	Susceptible to proteases

Table 3. Principal groups of Gram negative AMPs

target cell, and the rest of the protein has the lethal and immunity activities. Pyocin genes from *P. aeruginosa* PAO1 strain are found in the chromosome, are present as a group of 16 open reading frames, of which 12 are analogous to bacteriophage genes (Riley & Wertz, 2003; Williams et al., 2008). Microcins are encoded in plasmids or the chromosome; a typical gene clusters include the microcin precursor, the self-immunity factor, the secretion proteins and frequently the post-translational modification enzymes (Duquesne et al., 2007).

3.2 Synthesis and AMPs secretion from Gram negative bacteria

The production of colicins is performed under stress, reason why it is mediated by the SOS regulon (Gillor et al., 2008). The number of cells producing colicin in culture is very small, but the proportion increases when cells are exposed to stressors such as mitomycin and UV light (Jack et al., 1995). Pyocin synthesis in *P. aeruginosa* PAO1 occurs in a similar way. Synthesis starts when the stressor (which could cause damage to DNA) stimulates the expression of the RecA protein, whose main function is the repair of damaged DNA and to degrade the repressor protein (PRTR) to initiate the expression of the *prtN* activator gene; the PrtN protein then activates the expression of genes that codify for pyocins (Waite & Curtis, 2009). Microcins are also synthesized under stress conditions like a pro-microcin that is secreted to the medium after suffering a cut of 15 to 37 amino acid residues to release the active microcin; only the MccC7/C5 AMP from *E. coli* does not undergo this change (Duquesne et al., 2007; Novikova et al., 2007).

3.3 Gram negative AMPs action mechanisms

Colicins generally present three action mechanisms: some of them form pores or ion channels in the membrane, others have nuclease activity (colicin E2 and pyocin S3), others inhibit the synthesis of macromolecules (colicin E3), or as in the case of microcin, the action mode depends upon the organism that it is acting on. Microcin J25 acts on *E. coli* inhibiting RNA polymerase, while on *Salmonella enterica* forms pores in the membrane (Pugsley, 1984; Craik et al., 2003).

AMPs whose action is to form pores in the membrane destroy the organism by altering the membrane permeability, affecting the normal flow of ions like potassium, magnesium, sodium and chloride, as well as inhibiting ATP synthesis through the dissipation of the membrane electric potential and of the pH gradient. Examples of these AMPs are: glycinecin A from *Xanthomonas campestris*; A, E1, K, Ia and Ib colicins from *E. coli*; pyocin S5 from *P. aeruginosa* and xenocin from *Xenorhabdus nematophila* (Pham et al., 2004; Cascales et al., 2007; Singh & Banerjee, 2008; Zhang et al., 2010). Once released, some AMPs are attached to a membrane receptor present in the target cell, afterwards enter to the cell, usually helped by Tol-like proteins, and finally they may have access to intracellular targets (Lazaroni et al., 2002; Singh & Banerjee, 2008).

The AMPs that have nuclease activity enter to the cell and bind to tRNA or rRNA and break it at specific sites, thus inhibiting protein synthesis. Also, several AMPs can degrade nucleic acids without any specificity, for example: colicins E5, D and E7, and pyocins S1, S2, S3, S4 and AP41 (Masaki & Ogawa, 2002; Michel-Briand & Baysse, 2002; Hsia et al., 2005).

In the case of microcins, the facts that have a great diversity of post-translational modifications suggests that also have a great variety of action mechanisms; however, they show the typical nuclease and pore-formation mechanisms, although the latter is related to the production of siderophores. This dual mechanism of siderophore production and pore formation has been found in some microcins such as MccE492, produced by *Klebsiella pneumoniae* RYC492. The mechanism works as follows: the bacteria produces the siderophore to chelate environmental Fe^{3+}, thus preventing its use by other microorganisms; afterwards the siderophore undergoes post-translational modification and creates a glycopeptide capable of forming pores in the membrane of competing bacteria (Thomas et al., 2004; Duquesne et al., 2007; Nolan et al., 2007; Mercado et al., 2008).

3.4 AMPs resistance from Gram negative bacteria

Resistant mechanisms for Gram negative AMPs, different to self-immunity, have been described. It has been found some strains of *E. coli* resistant to others *E. coli* colicins, which have a Tol or Ton mechanisms altered, but is very specific and only works with the specific colicin. These resistant strains have been used to study the Tol and Ton mechanism (Braun et al., 1994). The pyocin resistant strains of *Neisseria gonorrhoeae* and *Haemophilus ducreyi*, have been found to be associated with structural differences in the outer membrane lipooligosaccharides in both species (John et al., 1991; Filiatrault et al., 2001). An *E. coli* K12 microcin resistant has been found, this strain possess a YojI protein which works as microcin J25 efflux pump (Socias et al., 2009). These examples show the variety of mechanisms displayed by bacteria to counteract the AMPs activity.

3.5 Potential Gram negative AMPs applications in biotechnology and biomedical therapies

The consumption of AMPs producing bacteria or the consumption of the purified peptides can be useful in establishing probiotic microorganisms in the gastrointestinal tract of humans and animals, which can lead to health improvements (Gillor et al., 2009). It has been found that in cystic fibrosis patients with an *P. aeruginosa* infection this organism produces pyocins that inhibit the growth of its closest competitors, so it could also be used as a therapeutic agent in these kind of patients and minimize the effects of the infection, that besides rooting out other susceptible. *P. aeruginosa* strains, also has an effect on *Haemophilus*,

Neisseria and *Campylobacter*. Regarding the latter, peritonitis treatment in mice has been successful (Scholl & Martin, 2008; Waite & Curtis, 2009; Williams et al., 2008). In other studies, colicin E1 has shown to inhibit the growth of *E. coli* O157:H7 *in vitro*, and the next step is to try it in meat and in the feeding of cattle to avoid the growth of *E. coli* O157:H7 in the gut (Callaway et al., 2004). The pyocin R-Type is studied as an antibiotic against *E. coli, Salmonella, Yersinia pestis and Pseudomonas* species by AvidBiotics Corp., with the name "Avidocin™ Proteins", but there is not still commercially available.

4. Animal and plant AMPs

As part of the defense mechanisms of multicellular organisms it can be found the production of compounds to eliminate invading microorganisms. Among these AMPs stand out; they are components of the innate immune response in higher eukaryotes. AMPs are mostly small, amphipathic and cationic peptides that possess diverse functions in addition to their antimicrobial properties. Currently, there have been over 1500 different AMPs described (Guaní-Guerra et al., 2010). Because of their great diversity, AMPs classification in higher eukaryotes has been hampered; however, five groups have been established based on their amino acid sequence and structural conformation; whereas in plants 10 families have been classified. Here are some general aspects of AMPs produced by animals and plants, emphasizing their action mechanism and their therapeutic and biomedical properties.

4.1 Animal AMPs

In animals, AMPs are produced at sites that are in constant contact with microorganisms, such as mucosal epithelial cells (respiratory, oral, genitourinary, gastrointestinal, etc.) or skin cells. In the case of insects, they are also produced in the fat body and hemocytes; and in vertebrates are produced and stored in monocytes, neutrophils, and mast cells, which constitute some of the non-oxidative effector mechanisms against potential pathogens. Animal AMPs can be produced constitutively or in response to infection (Brogden, 2005).

4.2 Animal AMPs classification

AMPs diversity is so large that their classification has been held back; however, five main groups are proposed which consist of those found in plants, vertebrates and invertebrates. These are described in Table 4, and the main representatives of the groups mentioned. Briefly, a group comprises anionic peptides including small peptides rich in glutamic and aspartic acid; a second group contains short cationic peptides (<40 residues) which lack cysteines and that in some environments adopt certain α-helical structures; a third group includes cationic peptides rich in various amino acids. There is a fourth group of anionic and cationic AMPs that present several cysteine residues, and therefore form disulfide bonds and stable α-sheets. These include most of the AMPs produced by plants as described below. Finally, there is a fifth group containing anionic and cationic peptides, which are fragments of larger proteins.

4.3 Plant AMPs

Plant AMPs are part of the defense mechanisms of these, they may be expressed constitutively or can be induced in response to a pathogen attack, and although lack of the sophistication of vertebrate adaptive immunity, they offer "fast" protection against pathogens. Compared with the production and action of secondary metabolites, AMPs can

Group	Representative AMPs	Source
Anionic peptides	Dermacidin Maximin H5	Human sweat glands Amphibians
Linear cationic peptides with α-helical structures	Melittin Magainin 2 Cecropin 37 Dermaseptin Cathelicidin LL37	Bee venom Amphibian skin Insects Amphibian skin Humans
Cationic peptides rich in certain amino acid residues	Histatin-5 (histidin rich) PR-39 (proline and arginine rich) Indolicin (triptophan rich)	Human saliva Pig neutrophils Cattle
Anionic and cationic peptides that contain cysteine and form disulfide bonds	Brevinin (1 S-S bond) Protegrin (2 S-S bonds) α and β defensins (3 S-S bonds) Defensins and Thionins (>3 S-S bonds) Drosomycin (>3 S-S bonds)	Amphibians Pigs Mammals (α and β), avians (α) Plants *Drosophila melanogaster*
Cationic and anionic peptides that are fragments of larger proteins	Lactoferricin from lactoferrin	Bovine milk

Table 4. Animal and plants AMPs classification based on amino acid composition, net charge and secondary structure (Epand & Vogel, 1999; Bradshaw, 2003; Brogden, 2005)

be released immediately after the infection is produced; they are expressed by a single gene and therefore require less biomass and energy expenditure (Thomma et al., 2002; Lay & Anderson, 2005). Most of characterized plant AMPs to date have a molecular weight in the range of 2 to 10 kDa; are basic and contain 4, 6, 8 or 12 cysteines that form disulfide bonds, giving them structural and thermodynamic stability (García-Olmedo et al., 2001; Lay & Anderson, 2005)

4.4 Plant AMPs classification

Plant AMPs are classified based on the identity of their amino acid sequence and the number and position of cysteines forming disulfide bonds. So far, 10 families have been described in plants, these are listed in Table 5 (García-Olmedo et al., 2001; Lay & Anderson, 2005). These include lipid transfer peptides (LTPs), thionins, defensins, hevein and knottin like proteins, as well as antimicrobial proteins isolated from *Macadamia integrifolia* (MBP-1) and *Impatiens balsamina* (Ib-AMP). All these AMPs exert their effect at the plasma membrane of the microorganisms that they attack, although their action mechanisms vary depending on the family. The cyclotides are members of a recently discovered peptide family rich in cysteine, commonly found in the *Rubiaceae*, *Violaceae* and *Cucurbitaceae* families; they present antibacterial and antiviral activities, as well as insecticide properties; besides containing a

head-tail cyclic backbone and a knotted arrangement of three conserved disulfide bonds (Daly et al., 2009).

Family	Amino acid number	Disulfide bonds	Acitivity vs.
LTPs	90–95	3–4	Bacteria and fungi
Snakins	61–70	6	Bacteria and fungi
Defensins	45–54	4	Bacteria and fungi
Thionins	45–47	3–4	Bacteria and fungi
Hevein-like	43	4	Gram (+) bacteria and fungi
Knottin-like	36–37	3	Gram (+) bacteria and fungi
Shepherins**	28–38	0 (linear)	Bacteria and fungi
MBP-1*	33	2	Bacteria and fungi
Cyclotides	29–31	3	Bacteria, viruses and insects
Ib-AMP*	20	2	Gram (+) bacteria and fungi

Table 5. Plant AMPs families (Lay & Anderson, 2005; García-Olmedo et al., 1998; Daly et al., 2009). * One member family; **two member family, which are derived from a polypeptide precursor

Thionins were the first AMPs whose antimicrobial activity against plant pathogens was demonstrated *in vitro* (García-Olmedo et al., 2001). This class of molecules has been found in various plant tissues, such as the seed endosperm, the stem and roots; they present a three-dimensional structure that can be represented by gamma letter (γ), where the vertical portion consists of a pair of antiparallel α-helices and the short horizontal arm consists of an antiparallel β-sheet (Thevissen et al., 1996). Thionins belong to a small group of basic peptides rich in cysteine that are toxic to bacteria and phytopathogenic fungi (Vignutelli et al., 1998; Zasloff, 2002). It has been suggested that toxicity requires the electrostatic interaction of the thionins with the negative charges of the membrane, causing the formation of pores (Thevissen et al., 1996).

Plant defensins are AMPs with an approximate molecular weight of 5 kDa, they are composed of 45 to 54 amino acids; they are basic and typically have eight cysteines. γ-purotionina (γ-1P) and γ-hordotionina (γ-1H) were the first isolated defensins, which were obtained from wheat and barley grains, respectively. These AMPs have been found in all studied plants, even it is hypothesized that they are ubiquitous in the plant kingdom. They have been isolated from sorghum, pea, tobacco, potato, petunia, beet, radish and several members of the *Brassicaceae* family (García-Olmedo et al., 1998), also from broad beans (*Vicia faba*) (Zhang & Lewis, 1997) and maize (*Zea mays*) (Kushmerick et al., 1998). AMPs have been detected in various tissues, mainly in those that are most exposed to contact with pathogens such as leaf primordia, the cells adjacent to the substomatal cavity, epidermis and stomata; in addition to seeds, leaves, pods, tubers, fruit, roots and bark (García-Olmedo et al., 1998; Lay & Anderson, 2005).

In relation to shepherins, they have been isolated from *Capsella bursa-pastoris*, they are rich in glycine and histidine and show activity against Gram negative bacteria and fungi (Park et

al., 2000). The snakins are peptides containing 12 cysteines, 6 disulfide bonds and have been isolated from potato. They present activity against plant pathogenic fungi and bacteria (Berrocal-Lobo et al., 2002).

4.5 Animal and plant AMPs genes

With regard to the genes that codify for animals and plants AMPs, they can be found in one or more copies with a variable intron number. In animals, it has been found that many of the genes that codify for AMPs have κB regulatory sequences, and therefore many of them are activated by NF-κB transcription factors, although it has also been reported that in higher eukaryotes there are other expression regulatory factors, such as the hypoxia-inducible factor (HIF), which regulates the expression of cathelicidins in mammals (Zarember & Malech, 2005; Hölzl et al., 2008), and the activator protein 1 (AP-1) transcription factor that regulates the expression of mammalian defensins (Wehkamp, 2004).

4.6 Animal and plant AMPs posttranslational modifications

Most studied AMPs are the product of larger proteins that contain a signal peptide, a pre-domain and a region corresponding to the mature peptide. The presence, length and relative position of these three regions varies among the different AMPs families, and only the mature peptide is the one that interacts with microorganisms (Lay & Anderson, 2005). They can also show modifications such as glycosylation, circularization, amidation of the ends and amino acid modification including D-amino acids (Boman, 1995; Nissen-Meyer & Nes, 1997).

4.7 Animal and plant AMPs action mechanisms

The nature of AMPs, based on their amino acid composition, charge and size allows them to be easily inserted into the lipid bilayer membranes of microorganisms. The general mechanism by which AMPs damage plasma membranes is considered universal for all described peptides, and is based on electrostatic interactions. In the case of bacteria, the interaction of cationic AMPs with anionic membrane phospholipids (phosphatidylglycerol and cardiolipin), and with the phosphate groups of Gram negative lipopolysaccharide (LPS), as well as the interaction with teichoic acids in Gram positive bacteria, occurs through electrostatic mechanisms, constituting the first step of action. Subsequently, peptides that are in close contact with the bacterial cell must pass through the capsular polysaccharide or teichoic and lipoteichoic acids to interact with the plasma membrane. Once the peptides have contacted it they can interact with the lipid bilayer. The second step is the permeabilizaton of the membrane; this mechanism is given by the formation of pores in the membrane due to interactions and arrangements of the AMPs. This leads to cell lysis by osmotic shock (Ogata et al., 1992; Boman, 1995, 2003).

These mechanisms may vary depending on different AMPs types, their concentration and the organism with which they interact. Besides, recently novel action mechanisms have been described that include the synthesis inhibition of nucleic acids, proteins, the cell wall, as well as the activity inhibition of some other enzymes (Bradshaw, 2003; Murray & Liu, 2008). The mechanisms related to cell membrane disruption and to intracellular target interactions are described below.

The AMPs interaction with membranes has been studied mainly in cationic peptides with α-helical structures. Although the interaction mechanism may be different for each type of peptide, their main action involves the instability of the outer membrane, translocating it

through the outer bilayer (Bradshaw, 2003; Téllez & Castaño, 2010); these mechanisms are explained in the "barrel-stave", "toroidal pore", "carpet", "molecular electroporation" and perforation of the" lipid raft" models, which are described below.

4.7.1 "Barrel-stave" model

This model proposes that initially, a group of cationic AMPs molecules with α-helical structures interact with each other on the surface of the plasma membrane to form a complex. Subsequently, the peptides are oriented perpendicular to the plane of the membrane allowing the hydrophobic region of the peptide to interact with the hydrophobic region of the bilayer, while the hydrophilic surface of the peptide is oriented inwards, forming a hydrophilic channel that expands along the membrane. In this way, the formed protein complex behaves as a pore inserted into the membrane. The formation of these channels causes alterations in the membrane potential, provokes the output of solutes and eventually results in cell lysis (Zhao et al., 2003) (Figure 3).

4.7.2 "Carpet" model

In this model it is proposed that cationic AMPs bind to the phospholipids in the outer layer of the membrane covering the bilayer as a "carpet", but without inserting themselves in it. At the beginning of the interaction, the peptides orient themselves parallel to the membrane. When the peptide reaches a certain critical concentration, the monomers rotate and reorient towards the hydrophobic core of the membrane causing the formation of micelles and the collapse of the membrane (Shai, 1995). The early stages of the AMP interface with the membrane are based on electrostatic interactions between the peptide positive charges and the negative charges of the membrane phospholipid heads (Shai, 1995, 1999); while pore formation is mainly governed by interactions between the hydrophobic region of the AMP and the hydrophobic center of the bilayer (Papo & Shai, 2003). The peptides that are characterized by having a "carpet"-type action mechanism have a low affinity for zwitterionic lipids in comparison with acidic lipids (Zhao et al., 2003). This model describes the action mechanism of most cationic AMPs, including dermaseptin from the skin of amphibians and insect cecropin. The "carpet" model (Figure 4) may explains the action mechanism of peptides with a size of less than 23 or 24 amino acid residues that do not cross the plasma membrane and whose action mechanism cannot be explained by the "barrel-stave" model (Zhao et al., 2003).

4.7.3 "Toroidal pore" model

The "toroidal pore" model explains the action mechanism of cationic peptides with α-helical structures and from those that form disulfide bonds. Initially, the peptide orients itself parallel to the plane of the plasma membrane and binds to the region of the phospholipid polar heads in a functionally inactive state. When the threshold of a peptide-lipid molar ratio is exceeded (e.g., 1:30 for magainin 2), the peptides are reoriented perpendicular to the plane of the bilayer, and in conjunction with several surrounding lipids they invert themselves towards the interior of the membrane's hydrophobic region. This forms a "dynamic supramolecular peptide-lipid complex", which causes the irreversible rupture of the membrane. The transition between the inactive and active state of the peptide bound to the membrane depends on AMPs concentration and the phospholipid composition of the bilayer (Huang, 2000).

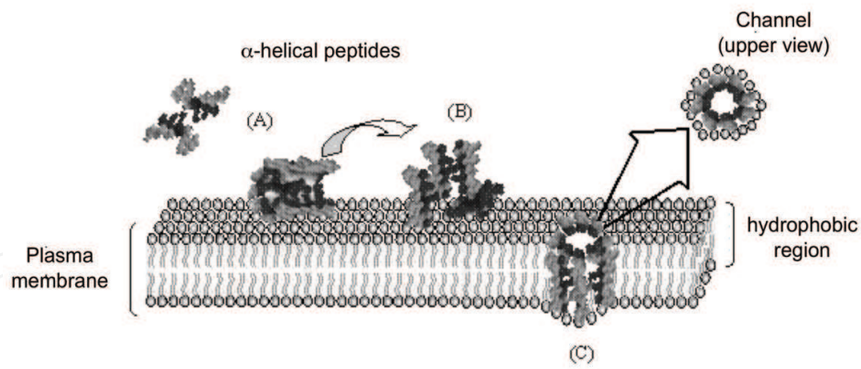

Fig. 3. Schematic representation of the "barrel stave" model explaining the interaction of antimicrobial peptides with bacterial membranes. In a first step (recruitment), the peptide monomers are joined together on the surface of the outer membrane of the bilayer. This process is governed primarily by the interaction of the peptide hydrophilic regions (shown in black), the recruited peptides are oriented parallel to the plane of the bilayer (panel A), when sufficient peptides are recruited (at least three of them) the peptide complex undergoes a perpendicular re-orientation to the plasma membrane (panel B), and finally the complex enters through the hydrophobic region of the bilayer (inset), forming a channel (panel C). Modified from Zhao et al., 2003

According to this model, the pores are formed by rows of lipids interposed to the peptides, which are oriented perpendicularly to the surface of the membrane, allowing the interaction of the hydrophilic regions of the pore with the polar heads of the phospholipids; which causes the lipid heads and the polar face of the α-helix, in the case of cationic peptides, to become oriented towards the pore's interior. As a result, the outside and interior faces of the bilayer become a continuous layer that delimits the interior of the pore. The newly formed pore allows for a coupled lipid and peptide transport across the bilayer with an increase of transmembrane movement of phospholipids ("flip-flop") and the orientation of the peptide monomers towards the interior of the bilayer. This arrangement differs from the classical channel depicted in the "barrel-stave" model (Figure 5); where interactions occur mainly between the hydrophobic face of the pore and the acyl chains of the bilayer's lipid core (Zhao et al., 2003). The magnitude, duration and required concentration for pore formation depends on the peptide, but is generally considered that the multipore state is the most stable structure and is formed when high concentrations of the peptide exist. However, individual pores may have a short lifetime and allow ion diffusion (Matsuzaki et al., 1997).

4.7.4 "Molecular electroporation" model

In this model, cationic AMPs are associated to the bacterial membrane generating an electric potential difference across it. The pore is generated when the potential difference reaches 0.2 V (Murray et al., 2008).

4.7.5 "Lipid raft" perforation model

This model proposes that the binding of an amphipathic AMP causes a mass imbalance and therefore an increase in the curvature of the membrane, which provides sufficient force to it to translocate through itself. Since AMPs self-associate, in this model they would sink into the membrane, generating a transient pore in which the peptides would be in both sides of it

(Murray & Liu, 2008). Moreover, there is growing evidence that indicates that AMPs have intracellular targets in addition to their plasma membrane interactions, because targets have been identified within microbial cells, and also because this mechanism explains why AMPs can enter the microbial cell without affecting its outer structure by passive transport (Nicolas, 2009).

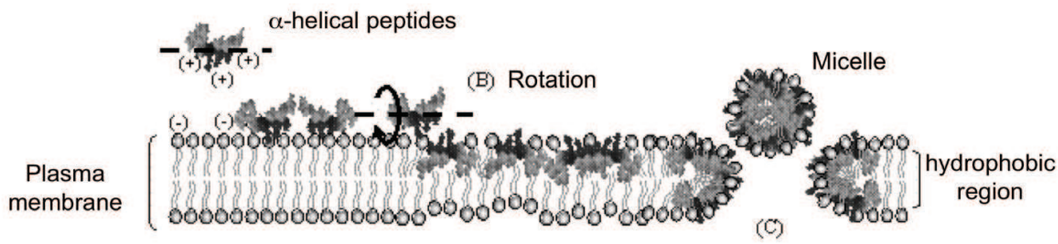

Fig. 4. Schematic representation of the "carpet" model explaining the interaction between antimicrobial peptides and bacterial membranes. This model describes the interaction that occurs between the positive charges of the α-helical cationic peptides and negatively charged polar phospholipid heads, which are oriented towards the outside of the membranes. Bound peptides remain parallel to the outer membrane of the bilayer (panel A), when they reach a critical concentration, the peptides rotate on their axis, causing the phospholipids bound to them to redirect (panel B), this shift produces the collapse of the structure of the plasma membrane and the formation of micelles with a hydrophobic core, forming a pore in the membrane (panel C). Modified from Zhao et al., 2003

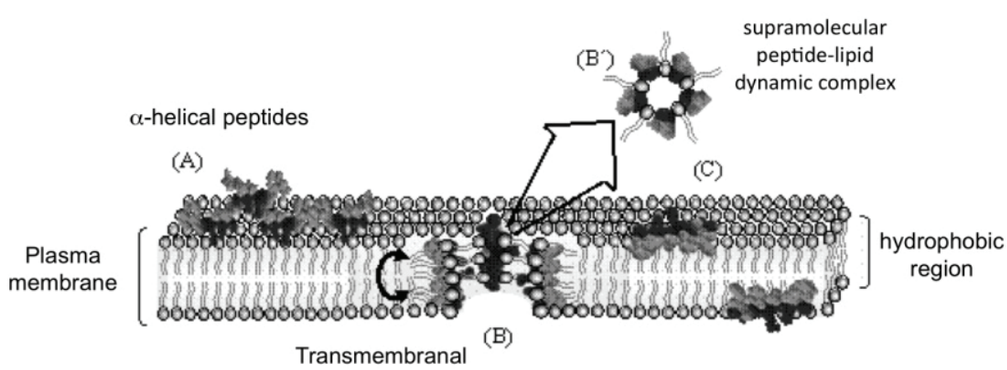

Fig. 5. Schematic representation of the "toroidal pore" model describing the interaction of antimicrobial peptides with bacterial membranes. This model, also known as a "two stage" model, describes the transition of the peptide from an inactive state to an active state. At low concentrations (inactive state), the peptides are oriented parallel to the plane of the bilayer (panel A). When they reaches a critical concentration, the peptide molecules are reoriented perpendicularly penetrating the hydrophobic region of the bilayer (active state) and together with some lipid molecules they adopt a multipore transitional state, known as a supramolecular peptide-lipid dynamic complex (panel B '), this produces the irreversible rupture of the plasma membrane and an increase in the "transmembranal movement" of lipids (two-headed arrow) (panel B). As a result of this increased "transmembranal movement" of lipids an orientation of the peptide molecules towards the inner layer of the bilayer may occur (panel C). Modified from Zhao et al., 2003

Two general mechanisms have been proposed to describe the process by which AMPs enter microbial cells: 1) spontaneous assisted translocation by lipids, and 2) a stereospecific receptor-mediated endocytosis. These internalization mechanisms vary depending on the peptide type and the target cell. In addition, the AMPs amino acid composition plays a crucial role in the internalization, since they are composed mainly of basic amino acids (principally arginine), AMPs can interact in a better way with membrane lipids allowing them to pass inside (Nicolas, 2009).

Once AMPs access the interior of the microbial cells, they interfere in metabolic functions such as: cytoplasm alteration, intracellular content agglutination, signaling pathways modification, regulation of transcription and inhibition of the transcription process, cell wall synthesis, nucleic acid synthesis, protein synthesis or enzyme activity (Brogden, 2005).

4.7.6 Other plant and animal AMPs action mechanisms

It has been reported that some AMPs from plants and insects carry out their effects through specific receptors localized in the membranes of some fungi. Such is the case of plant defensins RsAFP2 and DmAMP1 from *Raphanus sativus* and *Dahlia merckii* respectively, and the insect defensin heliomicin from *Heliothis virescens*; which interact with specific sphingolipids of plant and animal pathogenic fungi (Thevissen et al., 2007).

Many antimicrobial peptides are ineffective in normal mammalian cells. This seems to be related mainly to the lipid composition of target membrane (i.e. fluidity, negative charge density and the presence or absence of cholesterol), and to present a highly negative transmembrane electric potential (Nicolas, 2009). In tumor cells, AMPs interact with the membrane of cancer cells, which contain a small amount of phosphatidylserine giving them a greater negative charge compared to normal cells. In addition, cancer cells contain O-glycosylated mucins that attract serines and threonines from the AMPs. Another possible explanation for the peptide interaction with cancer cells is the high number of microvilli present in them, compared to normal cells, which increases the bonding surface of cancer cell membranes for AMPs (Papo & Shai, 2005).

The action mechanism of AMPs may also vary depending on their concentration, for example, at high concentrations the peptides can "carpet" the plasma membrane quickly generating micelles, causing cell lysis. On the other hand, at low concentrations, AMPs can slowly form pores in the membrane, they can also insert their polar region between phospholipids through the membrane from side to side causing the thinning of it, or they can cross the cell membrane without causing damage and attack or block an intracellular target (Hancock & Rozek, 2002; Brogden, 2005). It has also been shown that some AMPs regulate diverse functions of innate immunity such as neutrophil, mast cell or monocyte chemotaxis; they induce phagocytosis, are involved in tissue repair and angiogenesis, they can show anti-inflammatory properties and in some cases stimulate the production of cytokines and increase vascular permeability (Nicolas, 2009; Téllez & Castaño, 2010; Hölzl, 2008).

4.8 Resistance mechanisms towards animal and plant AMPs

Although AMPs production is an essential component of the plant and animal immunity, microorganisms, particularly bacteria, have developed various resistance mechanisms to them. These include mechanisms against AMP adhesion and insertion, as well as mechanisms that modify membrane permeability. In this sense, some bacteria have

developed modifications in the net charge on their surface, changes in membrane proteins, proteolytic enzyme production, removal of AMPs by transporters, etc. (Brogden, 2005).

4.9 Potential application of plant and animal AMPs in biomedical therapies

The potential usefulness of plant and animal AMPs clinical purposes resides in their use as antimicrobial agents, alone or in synergy with existing antibiotics. Similarly they can be employed as immunomodulatory agents or bacterial toxin neutralizers. Because many of them have low toxicity towards normal eukaryotic cells, but not for tumor cells, their use as anticancer drugs has been considered (Schweizer, 2009).

AMPs offer a good alternative for treating infections in relation to conventional antibiotics based on their broad spectrum activity and quick efficiency. However, very few plant and animal AMPs or synthetic derivatives of these have applications in clinical trials (Gordon et al., 2005). This follows the fact that they are susceptible to proteolysis, and that because of their chemical characteristics, their activity depends on the serum concentration of salts, or the pH of the medium in which they occur. For this reason the most promising AMPs in clinical evaluations are the ones that apply topically (Hancock & Sahl, 2006).

However, despite promising AMPs application, there are none currently approved for human use by the Food and Drug Administration (FDA). Only an AMP with topical application has shown efficiency in Phase III trials: AMP MX-226 (Omiganan pentahydrochloride, 1% gel; Migenix Laboratories), a synthetic peptide based on bovine indolicin and developed to prevent infections caused by the use of catheters. AMP synthetic derivatives are based on modifications to their three-dimensional structure and their biochemical properties, in order to show more stability and activity in different environments (Hancock & Sahl, 2006; Marr et al., 2006; Téllez & Castaño, 2010). Additionally, currently the possibility of inducing the endogenous production of AMPs is being considered, such is the case for the administration of sodium butyrate to induce the production of intestinal AMPs for the treatment of infectious or inflammatory diseases (Guaní-Guerra et al., 2010).

In the case of plant biotechnology transgenic plants resistant to diseases and pests have been produced through the introduction of AMPs genes from other plant species or even human defensins have been expressed in experimental models, and a better response to the attack of fungal pathogens has been observed (Aerts et al., 2007). Moreover, through biotechnological approaches, plant defensins and thionins have been expressed in mammalian cells in our working group; which showed activity against bacteria, fungi and tumor cells (Anaya-López et al., 2006; Loeza-Ángeles et al., 2008).

Among the studies that have been done with transgenic animals, those that demonstrate the protection conferred by human lactoferrin expressed in bovine mammary gland, conveying a delayed onset of clinical signs and inflammation caused by intramammary bacteria, stand out (Simojoki et al., 2010). In addition, bovine lactoferricin has been used in aquaculture to produce fish resistant to various infections (Lin et al., 2010).

5. Conclusion

AMPs are structurally diverse molecules, whose characteristics place them as a current and potential alternative to combat infections caused by pathogens resistant to conventional

antibiotics. In addition, diverse AMPs have shown biological properties different to antimicrobial activity, which positions them as tools for new biomedical therapies such as the modulation of the immune response, improved conventional antibiotic treatments, development of anticancer and anti-inflammatory therapies, the regulation of blood pressure and other biotechnological developments. Therefore, AMPs benefits in the biomedical area are well known; however, for the therapeutic application to succeed there is a multitude of their effects remains to be studied, as well as their biological and chemical characteristics in order to elucidate their action mechanisms.

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