Journal of The Faculty of Science and Technology (JFST)
http://journal.oiu.edu.sd/index.php/JFST
https://doi.org/10.52981/jfst.vi8.1951
ISSN: 1858 - 6007
مجلة العلوم و التقانة – جامعة أم درمان الإسلامية – وزارة التعليم العالي و البحث العلمي - السودان

Review paper **JFST Issue No. 8 (2021) 1 – 16**

The Insects Antimicrobial Peptides' Properties, Importance and Mode of Action

Osama AO Elhag[1,*] Manal Ismaiel[1]

[1]Department of Biotechnology- Sc. & Tech-Omdurman Islamic University, Sudan.

Email address:

osamaomer98@yahoo.com ,*Corresponding author,

Phone: +249900089470

Abstract: Anti-microbial peptides Antimicrobial peptides (AMP) are widely recognized as promising alternatives to the current use of antibiotics and fungicides. There are many desirable features, such as low cost of production, rapidity, heat-tolerant, relatively broad antimicrobial spectrum and low toxicity to eukaryotic cells made of antimicrobial peptides to be a new alternative to the conventional antibiotic. Wildly distributed in insects, plants and animal, native antibiotic peptides have anti-fungal, anti-bacterial, anti-virus, anti-parasitic and anti-cancer effects. AMPs from insect are classically cationic and usually composed of less than one hundred amino acids. While their structures are diverse, the majority of the AMPs can be classified into a limited number of families. The most frequent structures are represented by peptides presuming the alpha-helical structure in organic solutions or disulfide-stabilized β-sheets with or without α-helical domains present. Despite an affluence of information on structural requirements for their antipathogens activity, the mode of action of these peptides is not yet completely understood, therefore an understanding of Insect AMPs mechanism of action on pathogens will develop their uses as therapeutics means. Here we aim to have a comprehensive review of the research work reveal the mode of action of antimicrobial peptides from insects as well as shed the light on their therapeutic applications.

Keywords: Insect antimicrobial peptides; Innate immunity, AMP applications, Mode of action.

INTRODUCTION

Insects represent the biggest class within the animal kingdom in terms of species number (Chernysh et al, 2002). More than one million of insect species have been described and it is estimated that an equivalent number of species remains to be identified. (Chernysh et al, 2002 and Wang & Lai, 2010). The insect's resistance to pathogens has certainly contributed

to their intense proliferation and diversity (Vilmos, 1998). The insect are lacking the specific immune system as found in the higher animals, this make insect developed effective and complex innate immune system obliviously differ from the vertebrate's adaptive system (Vilmos, 1998). The Insect antimicrobial peptides are a key factor of the insects' immune system they are mainly synthesized by the fat body a large biosynthetic organ, functionally similar to the mammalian liver (Sondergaard, 1993).

Antimicrobial peptides (AMPs) have a variety of interesting biological functions including antibacterial, antifungal, antiparasitic, antitumoral, and antiviral activities. (Chen et al, 2012, Hale & Hancock, 2007and Yeaman & Yount, 2003).

As significant components of the innate immune system against infectious agents, AMPs are widely expressed in nature from insects and plants to highly evolved animal species with more complex immune systems. So far, hundreds of antimicrobial peptides have now been reported according to the antimicrobial Peptide Database. Brahmachary et al, 2004 and Wang & Wang, 2004)

while they possess common properties such as small size, an overall positive charge and amphipathicity in hydrophobic enviroment, most AMPs from different organisms can be broadly divided into four groups based on common secondary structure motifs: amphipathic α-helices (Bulet et al, 1991) intramolecular disulfide bond antibacterial peptide (Hoffmann et al, 199) the glycine rich peptides (Rees et al, 1997) and the proline rich peptides (Otvos, 2002). On the other hand The mechanisms of action by which antimicrobial peptides agents destroy their targets are highly complex and non-identical (Yeaman & Yount, 2003), counting membranes disrupt, targeting cytoplasmic components and interfering with metabolism (Wimley, 2010). Boman's and his group successfully purified an AMP, which obtain from hemolymph of immunized pupae of Hyalophora cecropia is the first AMP has been identified (Hultmark et al.1980). Many AMPs that are isolated from different insect species (Cociancich et al, 1994) are classified depending on their homology into several families, namely the cecropins, Boman & Hultmark, 1987 and Dickinson et al, 1988), attacins, (Casteels et al, 1990 and

Kockum et al, 1984) lysozymes (Hultmark et al. 1980 Engstrom, 1985) defencins (Dimarcq et al, 1990) and dipteracins. Wicker et al, 1990). All five antibacterial protein groups have been isolated from dipteran insects (Hultmark, 1993). The Drosophila having seven distinct groups of AMPs including (cecropins, drosocin, attacins, dipteracins, defensin, drosomycin and metchnikowins) which identified and characterized by whole genome microarray analysis (Irving et al. 2001).

In this review we will concentrate on the development of antimicrobial peptides from insect into useful therapeutic agents and their application to agriculture and disease hindrance. This will be followed by a description the mode of action of antimicrobial peptides against it is target pathogens.

APPLICATIONS OF ANTIMICROBIAL PEPTIDES

Currently, the need for safe and effective antimicrobial peptides agents increases in parallel with emergence of many antibiotic resistant strains as consequence of excessive use and widespread of the antibiotics (Seshadri et al, 2012). Many desirable features, such as low cost of production, Rapidity, heat-tolerant, relatively broad antimicrobial, spectrum and low toxicity to eukaryotic cells made of antimicrobial peptides to be a new alternative to the conventional antibiotic (Yeaman & Yount, 2003). They may play their role as antimicrobials or therapeutic agents by different ways. Firstly, they can be used as single antimicrobial agents, such as the traditional antibiotics. Secondly, they can be combined with other antimicrobial agents to improve the effectiveness of antimicrobial activity. Thirdly, they are able to augment the patient's innate immune system. Finally, they can be applied as the septic shock endotoxins- neutralizing agents (Gordon et al, 2005). To date, the use of AMPs as stand alone antimicrobial agents has received the most attention. On the other hand, apply of endotoxin-neutralizing agents as adjuncts to the classical antibiotic therapy have also been searched (Gordon et al, 2005). A lot of bacterial pathogens are known as resistant to current antibiotics were found to be responsive to antimicrobial peptides isolated from insects.

Cecropins, the polypeptides originally found in cecropia moth, Hyalophora cecropia (Steiner et al, 1981) were thought to be primarily responsible for antibacterial activity against many kinds of Gram positive and Gram negative bacteria (Hultmark et al, 1982) as well as fungi, when it is administration of cecropine in amount of 25 to 100 mg/ml unable it to cause fungicidal effects against the Aspergillus pathogenic species (DeLucca et al,.1997). F.moniliforme and F.oxysporum were particularly sensitive to cecropin A, with entire killing attained at 12.4 mg/m. (DeLucca et al,.1997). Gram-positive bacteria are highly affected by Insect defensins (Hetru et al, 2003), including Staphylococcus aureus the human pathogenic bacteria, whereas they do not exhibit powerful activity against Gram-negative bacteria (Yamada, and Natori, 1994).

The drosomycin, an insect defensin which produced by Drosophila melanogaster having a considerable similarity to the plant antifungal peptides purified from seeds of Brassicaceae family members (Fehlbaum et al, 1994), structurally it is analogous to the radish antifungal peptide, Rs-AFP1, furthermore, it is principally effective against F. oxysporum isolates (Michaut et al, 1996). Study conducted by Yamada and his team showed antimicrobial

activity of the defensin isolated from the Japanese rhinoceros beetle against the pathogenic Staphylococcus aureus strains resistant to antibiotics such as methicillin (Yamada et al, 2005). The D2A21 a cecropin analogue peptide was shown to be more effective in the cure of infected wounds than standard treatments, Long-term survival follow-up of rates receiving D2A21 indicate the survival time of this group was significantly prolonged with 100% of rats with infected wounds on the other hand survival rate in the control is only 50% (Chalekson et al, 2003). The antifungal peptides from Insect which include antifungal peptide, thanatin and holotricin3. Antifungal protein, a histidine-rich peptide that lead to cellular leakage, was isolated from the Sacrophaga peregrina's third instar larval hemolymph, and in vitro study confirmed, it is efficiency as lethal agent for C. albican (Matejuk et al, 2010). Thanatin, produced by Podisus maculiventris, is active against F. oxysporum and A. fumigates with no hemolytic effects (Fehlbaum et al, 1996). A peptide that rich in glycine and histidine which purified from the Holotrichia diomphalia larval's hemolymph, and named, Holotricin 3 has been proved to inhibit the C. albicans growth (Lee et al, 1995)

Halocidin is a heterodimer antimicrobial peptide isolated from a tunicate Halocynthia aurantium, was confirmed as active antimicrobial agent against resistant strains of Pseudomonas aerginosa and S. aureus. It is composed of an 18 residue domain connected to a 15 residue domain by a disulfide bond. The synthetic 18 residue heterodimer was found more active comparing to the natural one (Jang et al, 2002) Antimicrobial peptides have been extensively monitored for their use as dental and ophthalmic antimicrobials agents. As ophthalmic treatment the peptide can be used locally in the form of eye drops into the infected site while the quantity of active peptide can easily be augmented by additional dosing. Also it is having a promising application in the disinfection of contact lenses(Gordon et al, 2005). The cecropin analogues Shiva-11, hecate as well as D5C were tested for their capabilities to disinfect the contact lenses as well as the contact lens solutions. Shiva 11 and Hecate were able to slay bacterial isolates from contaminated contact lenses(Gordon et al, 2005). D5C was able to exponentially improve the ability of the existing contact lens sterilizing solutions to sterilize contact lenses (Sousa et al, 1996).

Chernysh and his collegues indicated that the Antimicrobial peptides secluded from insects have been shown promising role to prevent mortality in mice infected with influenza virus. These peptides were isolated from the carrion fly Calliphora vicina and named alloferons. It is competing the influenza virus hemaglutinin protein some basic structural elements. This makes the alloferon able to interrupt the viral assembly or even viral ligation to the cell (Chernysh et al, 2002). The altering the charge distribution of mellitin result in production of a highly successful mellitin derivative named hecate, with retaining its the three-

dimensional installation (Baghian et al, 199). Hecate also was demonstrated antiviral efficiency against herpes simplex virus-1; it can reduce plaque formation when administrated at relatively low concentrations. But it did not interfere with the virus's protein synthesize process. Furthermore, it was reported that Hecate was able to avoid (HSV-1) - induced cell fusion and virus propagation, with unknown cytotoxic effects (Baghian et al, 199).

It has been proved that cecropin beside the mellitin were able to reduce the HIV-1 multiplication infected cells. These peptides accomplish this by inhibiting the HIV-1's gene activity either by lowering the transcription or the number of viral gene output (Wachinger et al, 1998). The artificial peptides derivative from the beetle Allomyrina dichotoma defensin, have antimicrobial activities and anti-inflammatory effects, this mainly achieved by blocking the tumour necrosis factor-alpha (TNF-alpha) production the later effect was observed clearly by protect mice from endotoxic shock, the study hypothesized that the peptide preventing LPS interaction into it's receptors located on the macrophages surfaces, this was suggested a possible way by which the peptides prohibit TNF-α production (Koyama et al, 2006). In addition, the low molecular weight alloferon peptides isolated from C. vicina were found to motivate interferon production in mice as well as motivating mouse spleen lymphocyte cytotoxicity effects (Chernysh et al, 2002).

The Tachyplesin III peptide which Isolated from horseshoe crab was able to efficiently destroy P. aeruginosa the known multidrug resistant pathogen. This effect was significantly improved by used the Tachyplesin III in conjunction with conventional antibiotics (Cirioni et al, 2007). Tachyplesin has similarity to defensins by protect the mice from endotoxic shock caused by bacterial lysis (Cirioni et al, 2007). The hemipteran peptide thanatin was found highly effective against multidrug resistant isolates of Klebsiella pneumonia and Enterobacter aerogenes. These strains able to resist the antibiotics effect by altering their membrane's permeability, which allows them to eject antibiotics regardless of structure. The suggested possible mechanism by which tachyplesin III and thanatin augmented the antibiotic susceptibility of these resistant isolates must done by making the bacteria's membrane more porous to the antibiotics or interfering with the bacteria's ability to expel the cell membrane's specific character. The bacteria and tumor cell membranes are sharing common characteristic that both are negatively charged Leuschner and Hansel, 2004). This is due to The tumor cell membranes is characterized by a few number of negative phosphatidylserine, this let them 3-9% more negative comparing to the normal cells Zwaal and Schroit, 1997). Altering a few number of L-amino acids with D-enantiomers resulted in Mellitin derivatives production, the new peptides has a high efficiency with no hemolytic effects (Papo and Shai, 2003). One more mellitin analogue, hecate, was recored as a toxic to breast cancer cells (Leuschner et al, 2003). Creation of hecate hormone conjugates result in

the Hecate's effectiveness propagation, this was achieved by conjugating hecate to hormones, whose their receptors are located on the cancer cells surface such as the luteinizing hormone, by which the cell selectivity of hecate will be improved (Chernysh et al, 2002). Among the artificial homodimer peptide analogues derived from harmoniasin a defensin-like antimicrobial peptide which identified from the ladybug Harmonia axyridis, HaA4 has been found to have an efficient antibacterial activity with unknown hemolytic activity, recently, In-Woo Kim suggested that the HaA4 may use as effective therapeutic agent against human leukemia cell lines such as Jurkat cells and U937 (Kim, 2013).

MODES OF ACTION

Recently, it has become clear that AMP exert their effect by interacting with the anionic microbial surface and to insert into cytoplasmic membrane consisting of phospholipids. This action results in disruption of membrane integrity, like depolarization and pore formation. In addition to the membrane-active property, some insect AMPs have been recently reported to cause antimicrobial activity via different mechanisms including, Inhibition of cell respiration, inhibit the cell walls formation, deactivation of bacterial protein and induction of yeast apoptosis.

MEMBRANES DISRUPTIVE MECHANISM

AMP Interactions with the cytoplasmic membrane started when the cationic antimicrobial peptides ligation with the phospholipids, as long as the peptides: lipids ratio is low, the cationic antimicrobial peptides remain associated, parallel to the plane of the membrane inserted at the interface of the hydrophilic lipid head groups and the hydrophobic fatty acyl chain. However, as increases the peptide: Lipid ratio, these peptides become able to aggregate and /or reorient in the membrane and disrupt the membrane integrity (Hale and Hancock, 2007). This usually done through one of three proposed models; barrel-stave, carpet and toroidal pore model. In the barrel-stave model the membrane disruption achieved by forming a pore in the membrane by made up of bundles of helices as the result of peptide monomers association, while in the carpet model , the peptides assembly on the membrane surface and disrupt membrane structure via toroidal pores formation finally, toroidal pore model, where the peptides binding to the surface of the membrane and associate with lipid head groups causing a thinning of the membrane (Huang, 2000). The effect of tick defensin, a synthetic bactericidal on the Gram-positive bacteria M. luteus was confirmed by Transmission electron micrographs in which the lysis of the cytoplasmic membrane and leakage of cellular cytoplasmic contents was observed. These results suggest that the basic mechanism of defensin action is bacterial cytoplasmic membrane lysis (Shen et al, 2012).

The recombinant insect defensin from the blood of larvae of the flesh fly Phormia terranova mode of action against M. luteus has been addressed. It was shown that the defensin cause loss of cytoplasmic potassium, a partial depolarization of the inner membrane, a decrease in cytoplasmic ATP, and inhibition of respiration as consequence of disruptionthe permeability barrier of the cytoplasmic membrane of the bacteria by forming voltage-dependent channels in the bacteria (Cociancich et al, 1993). Study conducted by Geert van den and his colleagues found that the melittin causes two effects on liposomes composed of zwitterionic DOPC lipids. First, the leading to the vesicles content leakage as the result of peptide -pores formation. Second effect, protecting the membrane from leakage when the melittin attached to the membrane surface in an inactive conformation, thereby preventing other melittin molecules from inserting into the bilayer and hence (Van den Bogaart et al, 2008). Lysozymes are up regulated upon infection in the lepidopteran insects are muramidases that hydrolyse the β-1, 4- glycosidic linkage in the N-acetyl glucosamine and N-acetyl muramic acid residues in the peptidoglycan layer of the bacterial cell and cause their lysis (Daffre et al, 1994). Attacin is active against Gram negative bacteria by inhibiting the synthesis of its outer membrane protein whereas moricin increases their membrane permeability thereby kills Gram positive and negative bacteria (Hara et al, 1995). Study conducted by K Wang and his group showed that polybia-MPI targets at the membrane of bacteria, disrupts the integrity of membrane, and finally leads the leakage of cell contents of bacteria. In addition, the results of DNA binding properties of polybia-MPI showed that it did not bind with DNA and confirmed that polybia-MPI targets at cell membrane and exerts its antimicrobial activity (Wang et al, 2013).

Some others AMPs are killing the fungi by changing the membrane integrity, such as cecropins which found not toxic for mammalian cells at microbicidal dosage and have been administered safely to animals, they accomplished their effect by form the time-variant and voltage-dependent ion channels in planar lipid membranes when they are positively charged (Boman & Hultmark, 1987 and Christensen et al, 1988). Spinigerin produces by P. spiniger acts via membrane permeabilization in a similar manner to magainin 2, an antimicrobial peptide from Xenopus (Zasloff, 1987)

MEMBRANES NON-DISRUPTIVE MECHANISM

While most AMPs interact with and effect the integrity of microbial membranes, it is unclear if membrane permeabilization is always the lethal event or if the membrane is only the site of action. There are AMPs that may have alternate modes of action. Sarcotoxins II and β-defensin-3 both can inhibit the formation of cell wall to prevent bacteria from maintaining normal cell morphology, but has no effect on the already existing cell wall (Ando and Natori, 1988). The class of antimicrobial peptides secreted by insects, such as pyrrhocoricin,

apidaecin, and drosocin, are thought to kill bacteria by entering cells and inhibiting the molecular chaperone (DnaK et al, 2001)

Otvos and colleagues proposed that l-pyrrhocoricin binds predominately to a `non-conventional binding site' on DnaK, that is, the αE and αD helices of the multi-helical lid subdomain , and that l-PYR prevents DnaK's lid from opening and closing (Chesnokova et al, 2004). Thanatin does not permeabilize bacterial membranes but instead causes rapid agglutination of bacterial cells. An all D-enantiomer of the peptide has no antibacterial activity yet activity against filamentous fungi is unchanged (Fehlbaum et al, 1996). Study conducted by Fehlbaum, Bulet and Gudmundsson indicated that the Thanatin kills bacteria through inhibiting cell respiration, rather than affecting the cell membrane this was justified by the observation the outward flow of K+ coula not be detected when bacterial cells were treated with different concentrations of Thanatin, which suggesting the membrane was not the target of peptide, while increasing the Thanatin concentration (40 μmol/L) could weaken cell respiration after one hour treatment, and six hours later respiration entirely stop (Fehlbaum et al, 1996). Bobek found that the potent killing activity caused by MUC7 20-mer against a variety of fungi and both gram-positive and gram-negative bacteria is result of relevant to inhibiting cell respiration (Bobek and Situ, 2003). Hong RW et al, demonstrate that a nonlethal response with intracellular consequences in E. coli was encouraged by cecropin A antimicrobial peptide, this response takes the form of altered transcript levels for a distinct and rather small set of genes (Hong et al, 2003). Attacin could interfere the transcription of outer membrane protein gene to decrease the contents of these proteins, increasing membrane permeability to inhibit the bacterial growth (Harder et al, 2001). DNA, the genetic information carrier, plays an important role in the physiological processes. It has gained much of people’s interest on the interaction between AMPs and DNA. It was found out that some peptides could combine with bacterial DNA to affect the normal physiological function. AMPs could combine with pECP1 to influence its migration rate by gel mobility shift assay (Zhang et al, 1999). Hariton was found that peptides PVS4 (13) and RRS4 (13) could bind to cell nucleus of cancer, inducing DNA fragmentation preceding cell death. However, despite the structural similarity to magainin 2, Chan Bae Park et al, clarified that the buforin II bound to DNA and RNA of the cells more than 20 times robustly than magainin 2. These results indicate that buforin II inhibits the DNA and RNA functions by binding to them after passing the cell membranes, and causing the rapid cell death, which is completely different from that of magainin 2 although they are structurally comparable: a linear amphipathic a-helical peptide (Park et al, 1998).

DISCUSSION& CONCLUSION

As the extensive use of antibiotics in medicine, farming and food industry, there is an

increasing tendency of emergence of multi-drug resistant bacteria, which have made the conventional antibiotics useless or limited use. So new antibiotics are urgently needed recently and more attention consults to the native AMPs (Peters et al, 2010). with several desirable properties, such as heat-tolerance, relatively broad antimicrobial spectrum and low toxicity to eukaryotic cells (Zeitler et al, 2013), AMPs, especially the "food-derived antimicrobial peptides" may serve as a potentially significant group of food preservatives. To date resistance to AMPs is rare and AMPs are thought to be promising alternatives to conventional antibiotics (Upton et al, 2012). AMPs are an essential element of innate immunity which can quickly respond to different microbial pathogens. The insects, as an extensive source of AMPs, attract great attention of researchers in both understanding of the fundamental biology of the immune system and screening molecular templates for anti-infective drug design. In spite of a large number of AMPs have been recognized from diverse insect species, few information in terms of these peptides is available concerning their applications (Tian et al, 2010). Insects are vectors of a wide range of animal and human parasites like malaria, yellow fever, dengue fever and sleeping sickness, all of which are major causes of mortality many of these parasites are exposed to the action of antimicrobial peptides at some point during their life cycle (Lowenberger, 2001). This has led to research which ultimately aims to produce transgenic forms of insect vectors which will kill the parasite, thereby preventing the transmission to humans (Lowenberger, 2001).

In conclusion, Insect antimicrobial peptides are the important components of insect immune systems with the advantages of small molecule weight, heat-stability and broad antibacterial activity. Antimicrobial peptides from insects are may serve as effective weapons versus a broad range of infectious agent and are disseminated throughout the animal and plant kingdom, indicating that they are essential for the successful development of complex multi-cellular organisms. The problem of large scale production has been taken care by the growing advent of recombinant synthesis technology which is effective and cheaper. Hence, the present focus would be to identify more novel peptides, re-design the existing peptides to get rid of their toxicity and develop novel recombinant protocols to obtain greater yield of peptides at lower cost.

REFERENCES

Ando, K.; Natori, S. Inhibitory effect of sarcotoxin IIA, an antibacterial protein of Sarcophaga peregrina, on growth of Escherichia coli. *J. Biochem.***1988,** *103*, 735-739.

Baghian, A.; Jaynes, J.; Enright, F.; Kousoulas, K. G. An amphipathic alpha-helical synthetic peptide analogue of melittin inhibits herpes simplex virus-1 (HSV-1)-induced cell fusion and virus spread. *Peptides.***1997,** *18,* 177-183.

Bobek, L. A.; Situ, H. MUC7 20-Mer: investigation of antimicrobial activity, secondary structure, and possible mechanism of antifungal action. *Antimicrob Agents Chemother.***2003,** *47*, 643-652.

Boman, H. G.; Hultmark, D. Cell-free immunity in insects. *Annu. Rev. Microbiol.***1987,** *41*, 103-126.

Brahmachary, M.; Krishnan, S. P.; Koh, J. L.; Khan, A. M.; Seah, S. H.; Tan, T. W.; Brusic, V.; Bajic, V. B. ANTIMIC: a database of antimicrobial sequences. *Nucleic Acids Res.***2004,** *32*, D586-589.

Bulet, P.; Cociancich, S.; Dimarcq, J. L.; Lambert, J.; Reichhart, J. M.; Hoffmann, D.; Hetru, C.; Hoffmann, J. A. Insect immunity. Isolation from a coleopteran insect of a novel inducible antibacterial peptide and of new members of the insect defensin family. *J. Biol. Chem.***1991,** *266*, 24520-24525.

Casteels, P.; Ampe, C.; Riviere, L.; Van Damme, J.; Elicone, C.; Fleming, M.; Jacobs, F.; Tempst, P. Isolation and characterization of abaecin, a major antibacterial response peptide in the honeybee (Apis mellifera). *Eur. J. Biochem.***1990,** *187*, 381-386.

Chalekson, C. P.; Neumeister, M. W.; Jaynes, J. Treatment of infected wounds with the antimicrobial peptide D2A21. *J. Trauma.***2003,** *54*, 770-774.

Chen, C.; Hu, J.; Zhang, S.; Zhou, P.; Zhao, X.; Xu, H.; Yaseen, M.; Lu, J. R. Molecular mechanisms of antibacterial and antitumor actions of designed surfactant-like peptides. *Biomaterials.***2012,** *33*, 592-603

Chernysh, S.; Kim, S. I.; Bekker, G.; Pleskach, V. A.; Filatova, N. A.; Anikin, V. B.; Platonov, V. G.; Bulet, P. Antiviral and antitumor peptides from insects. *Proc. Natl .Acad. Sci. U S A.***2002,** *99*, 12628-12632.

Chesnokova, L. S.; Slepenkov, S. V.; Witt, S. N. The insect antimicrobial peptide, L-pyrrhocoricin, binds to and stimulates the ATPase activity of both wild-type and lidless DnaK. *FEBS Lett.***2004,** *565*, 65-69.

Christensen, B.; Fink, J.; Merrifield, R. B.; Mauzerall, D. Channel-forming properties of cecropins and related model compounds incorporated into planar lipid membranes. *Proc. Natl. Acad. Sci. U S A.***1988,** *85*, 5072-5076.

Cirioni, O.; Ghiselli, R.; Silvestri, C.; Kamysz, W.; Orlando, F.; Mocchegiani, F.; Di Matteo, F.; Riva, A.; Lukasiak, J.; Scalise, G.; Saba, V.; Giacometti, A. Efficacy of

tachyplesin III, colistin, and imipenem against a multiresistant Pseudomonas aeruginosa strain. *Antimicrob. Agents Chemother.***2007,** *51*, 2005-2010.

Cociancich, S.; Bulet, P.; Hetru, C.; Hoffmann, J. A. The inducible antibacterial peptides of insects. *Parasitol. Today.***1994,** *10*, 132-139.

Cociancich, S.; Ghazi, A.; Hetru, C.; Hoffmann, J. A.; Letellier, L. Insect defensin, an inducible antibacterial peptide, forms voltage-dependent channels in Micrococcus luteus. *J. Biol. Chem.***1993,** *268*, 19239-19245.

Daffre, S.; Kylsten, P.; Samakovlis, C.; Hultmark, D. The lysozyme locus in Drosophila melanogaster: an expanded gene family adapted for expression in the digestive tract. *Mol. Gen. Genet.***1994,** *242*, 152-162.

DeLucca, A. J.; Bland, J. M.; Jacks, T. J.; Grimm, C.; Cleveland, T. E.; Walsh, T. J. Fungicidal activity of cecropin A. *Antimicrob. Agents Chemother.***1997,** *41*, 481-483.

Dickinson, L.; Russell, V.; Dunn, P. E. A family of bacteria-regulated, cecropin D-like peptides from Manduca sexta. *J. Biol. Chem.***1988,** *263*, 19424-19429.

Dimarcq, J. L.; Zachary, D.; Hoffmann, J. A.; Hoffmann, D.; Reichhart, J. M. Insect immunity: expression of the two major inducible antibacterial peptides, defensin and diptericin, in Phormia terranovae. *EMBO J.***1990,** *9*, 2507-2515.

Engstrom, A.; Xanthopoulos, K. G.; Boman, H. G.; Bennich, H. Amino acid and cDNA sequences of lysozyme from Hyalophora cecropia. *EMBO J.***1985,** *4*, 2119-2122.

Fehlbaum, P.; Bulet, P.; Chernysh, S.; Briand, J. P.; Roussel, J. P.; Letellier, L.; Hetru, C.; Hoffmann, J. A. Structure-activity analysis of thanatin, a 21-residue inducible insect defense peptide with sequence homology to frog skin antimicrobial peptides. *Proc. Natl. Acad. Sci. U S A.***1996,** *93*, 1221-1225.

Fehlbaum, P.; Bulet, P.; Michaut, L.; Lagueux, M.; Broekaert, W. F.; Hetru, C.; Hoffmann, J. A. Insect immunity. Septic injury of Drosophila induces the synthesis of a potent antifungal peptide with sequence homology to plant antifungal peptides. *J. Biol. Chem.***1994,** *269*, 33159-33163.

Gordon, Y. J.; Romanowski, E. G.; McDermott, A. M. A review of antimicrobial peptides and their therapeutic potential as anti-infective drugs. *Curr. Eye Res.***2005,** *30*, 505-515.

Hale, J. D.; Hancock, R. E. Alternative mechanisms of action of cationic antimicrobial peptides on bacteria. *Expert Rev. Anti .Infect. Ther.***2007,** *5*, 951-959.

Hara, S.; Yamakawa, M. Moricin, a novel type of antibacterial peptide isolated from the silkworm, Bombyx mori. *J. Biol. Chem.***1995,** *270*, 29923-29927.

Harder, J.; Bartels, J.; Christophers, E.; Schroder, J. M. Isolation and characterization of human beta -defensin-3, a novel human inducible peptide antibiotic. *J. Biol. Chem.***2001,** *276*, 5707-5713.

Hetru, C.; Troxler, L.; Hoffmann, J. A. Drosophila melanogaster antimicrobial defense. *J. Infect Dis.***2003,** *187 Suppl 2*, S327-334.

Hoffmann, J. A.; Kafatos, F. C.; Janeway, C. A.; Ezekowitz, R. A. Phylogenetic perspectives in innate immunity. *Science.***1999,** *284*, 1313-1318.

Hong, R. W.; Shchepetov, M.; Weiser, J. N.; Axelsen, P. H. Transcriptional profile of the Escherichia coli response to the antimicrobial insect peptide cecropin A. *Antimicrob. Agents Chemother.***2003,** *47*, 1-6.

Huang, H. W. Action of antimicrobial peptides: two-state model. *Biochemistry.***2000,** *39*, 8347-8352.

Hultmark, D. Immune reactions in Drosophila and other insects: a model for innate immunity. *Trends Genet.***1993,** *9*, 178-183.

Hultmark, D.; Engstrom, A.; Bennich, H.; Kapur, R.; Boman, H. G. Insect immunity: isolation and structure of cecropin D and four minor antibacterial components from Cecropia pupae. *Eur. J. Biochem.***1982,** *127*, 207-217.

Hultmark, D.; Steiner, H.; Rasmuson, T.; Boman, H. G. Insect immunity. Purification and properties of three inducible bactericidal proteins from hemolymph of immunized pupae of Hyalophora cecropia. *Eur. J. Biochem.***1980,** *106*, 7-16.

Irving, P.; Troxler, L.; Heuer, T. S.; Belvin, M.; Kopczynski, C.; Reichhart, J. M.; Hoffmann, J. A.; Hetru, C. A genome-wide analysis of immune responses in Drosophila. *Proc. Natl. Acad. Sci U S A.***2001,** *98*, 15119-15124.

Jang, W. S.; Kim, K. N.; Lee, Y. S.; Nam, M. H.; Lee, I. H. Halocidin: a new antimicrobial peptide from hemocytes of the solitary tunicate, Halocynthia aurantium. *FEBS Lett.***2002,** *521*, 81-86.

Kim, I. W.; Lee, J. H.; Kwon, Y. N.; Yun, E. Y.; Nam, S. H.; Ahn, M. Y.; Kang, D. C.; Hwang, J. S. Anticancer activity of a synthetic peptide derived from harmoniasin, an antibacterial peptide from the ladybug Harmonia axyridis. *Int. J. Oncol.***2013,** *43*, 622-628.

Kockum, K.; Faye, I.; Hofsten, P. V.; Lee, J. Y.; Xanthopoulos, K. G.; Boman, H. G. Insect immunity. Isolation and sequence of two cDNA clones corresponding to acidic and basic attacins from Hyalophora cecropia. *EMBO J.***1984,** *3*, 2071-2075.

Koyama, Y.; Motobu, M.; Hikosaka, K.; Yamada, M.; Nakamura, K.; Saido-Sakanaka, H.; Asaoka, A.; Yamakawa, M.; Sekikawa, K.; Kitani, H.; Shimura, K.; Nakai, Y.; Hirota, Y. Protective effects of antimicrobial peptides derived from the beetle Allomyrina dichotoma defensin on endotoxic shock in mice. *Int. Immunopharmacol.***2006,** *6*, 234-240.

Kragol, G.; Lovas, S.; Varadi, G.; Condie, B. A.; Hoffmann, R.; Otvos, L., Jr. The antibacterial peptide pyrrhocoricin inhibits the ATPase actions of DnaK and prevents chaperone-assisted protein folding. *Biochemistry.***2001,** *40*, 3016-3026.

Lee, S. Y.; Moon, H. J.; Kurata, S.; Natori, S.; Lee, B. L. Purification and cDNA cloning of an antifungal protein from the hemolymph of Holotrichia diomphalia larvae. *Biol.Pharm. Bull.***1995,** *18*, 1049-1052.

Leuschner, C.; Enright, F. M.; Gawronska, B.; Hansel, W. Membrane disrupting lytic peptide conjugates destroy hormone dependent and independent breast cancer cells in vitro and in vivo. *Breast Cancer Res. Treat.***2003,** *78*, 17-27.

Leuschner, C.; Hansel, W. Membrane disrupting lytic peptides for cancer treatments. *Curr. Pharm. Des.***2004,** *10*, 2299-2310.

Lowenberger, C. Innate immune response of Aedes aegypti. *Insect Biochem. Mol. Biol.***2001,** *31*, 219-229.

Matejuk, A.; Leng, Q.; Begum, M. D.; Woodle, M. C.; Scaria, P.; Chou, S. T.; Mixson, A. J. Peptide-based Antifungal Therapies against Emerging Infections. *Drugs Future.***2010,** *35*, 197.

Michaut, L.; Fehlbaum, P.; Moniatte, M.; Van Dorsselaer, A.; Reichhart, J. M.; Bulet, P. Determination of the disulfide array of the first inducible antifungal peptide from insects: drosomycin from Drosophila melanogaster. *FEBS Lett.***1996,** *395*, 6-10.

Otvos, L., Jr. The short proline-rich antibacterial peptide family. *Cell Mol. Life Sci.***2002,** *59*, 1138-1150.

Papo, N.; Shai, Y. New lytic peptides based on the D,L-amphipathic helix motif preferentially kill tumor cells compared to normal cells. *Biochemistry.***2003,** *42*, 9346-9354.

Park, C. B.; Kim, H. S.; Kim, S. C. Mechanism of action of the antimicrobial peptide buforin II: buforin II kills microorganisms by penetrating the cell membrane and inhibiting cellular functions. *Biochem. Biophys. Res. Commun.***1998,** *244*, 253-257.

Peters, B. M.; Shirtliff, M. E.; Jabra-Rizk, M. A. Antimicrobial peptides: primeval molecules or future drugs? *PLoS Pathog.***2010,** *6*, e1001067.

Rees, J. A.; Moniatte, M.; Bulet, P. Novel antibacterial peptides isolated from a European bumblebee, Bombus pascuorum (Hymenoptera, Apoidea). *Insect Biochem. Mol. Biol.***1997,** *27*, 413-422.

Seshadri Sundararajan, V.; Gabere, M. N.; Pretorius, A.; Adam, S.; Christoffels, A.; Lehvaslaiho, M.; Archer, J. A.; Bajic, V. B. DAMPD: a manually curated antimicrobial peptide database. *Nucleic Acids Res.***2012,** *40*, D1108-1112.

Shen, L.; Liu, D.; Li, M.; Jin, F.; Din, M.; Parnell, L. D.; Lai, C. Q. Mechanism of action of recombinant acc-royalisin from royal jelly of Asian honeybee against gram-positive bacteria. *PLoS One.***2012,** *7*, e47194.

Sondergaard, L. Homology between the mammalian liver and the Drosophila fat body. *Trends. Genet.***1993,** *9*, 193.

Sousa, L. B.; Mannis, M. J.; Schwab, I. R.; Cullor, J.; Hosotani, H.; Smith, W.; Jaynes, J. The use of synthetic Cecropin (D5C) in disinfecting contact lens solutions. *CLAO J.***1996,** *22*, 114-117.

Steiner, H.; Hultmark, D.; Engstrom, A.; Bennich, H.; Boman, H. G. Sequence and specificity of two antibacterial proteins involved in insect immunity. *Nature.***1981,** *292*, 246-248.

Tian, C.; Gao, B.; Fang, Q.; Ye, G.; Zhu, S. Antimicrobial peptide-like genes in Nasonia vitripennis: a genomic perspective. *BMC Genomics.***2010,** *11*, 187.

Upton, M.; Cotter, P.; Tagg, J. Antimicrobial peptides as therapeutic agents. *Int. J. Microbiol.***2012,** *2012*, 326503.

Van den Bogaart, G.; Guzman, J. V.; Mika, J. T.; Poolman, B. On the mechanism of pore formation by melittin. *J. Biol. Chem.***2008,** *283*, 33854-33857.

Vilmos, P.; Kurucz, E. Insect immunity: evolutionary roots of the mammalian innate immune system. *Immunol. Lett.***1998,** *62*, 59-66.

Wachinger, M.; Kleinschmidt, A.; Winder, D.; von Pechmann, N.; Ludvigsen, A.; Neumann, M.; Holle, R.; Salmons, B.; Erfle, V.; Brack-Werner, R. Antimicrobial peptides melittin and cecropin inhibit replication of human immunodeficiency virus 1 by suppressing viral gene expression. *J. Gen. Virol.***1998,** *79 (Pt 4)*, 731-740.

Wang, K.; Yan, J.; Dang, W.; Liu, X.; Chen, R.; Zhang, J.; Zhang, B.; Zhang, W.; Kai, M.; Yan, W.; Yang, Z.; Xie, J.; Wang, R. Membrane active antimicrobial activity and molecular dynamics study of a novel cationic antimicrobial peptide polybia-MPI, from the venom of Polybia paulista. *Peptides.***2013,** *39*, 80-88.

Wang, Y. P.; Lai, R. [Insect antimicrobial peptides: structures, properties and gene regulation]. *Dongwuxue Yanjiu.***2010,** *31*, 27-34.

Wang, Z.; Wang, G. APD: the Antimicrobial Peptide Database. *Nucleic Acids Res.***2004,** *32*, D590-592.

Wicker, C.; Reichhart, J. M.; Hoffmann, D.; Hultmark, D.; Samakovlis, C.; Hoffmann, J. A. Insect immunity. Characterization of a Drosophila cDNA encoding a novel member of the diptericin family of immune peptides. *J. Biol. Chem.***1990,** *265*, 22493-22498.

Wimley, W. C. Describing the mechanism of antimicrobial peptide action with the interfacial activity model. *ACS Chem. Biol.***2010,** *5*, 905-917.

Yamada, K.; Natori, S. Characterization of the antimicrobial peptide derived from sapecin B, an antibacterial protein of Sarcophaga peregrina (flesh fly). *Biochem. J.***1994,** *298 Pt 3*, 623-628.

Yamada, M.; Nakamura, K.; Saido-Sakanaka, H.; Asaoka, A.; Yamakawa, M.; Yamamoto, Y.; Koyama, Y.; Hikosaka, K.; Shimizu, A.; Hirota, Y. Therapeutic effect of modified oligopeptides from the beetle Allomyrina dichotoma on methicillin-resistant Staphylococcus aureus (MRSA) infection in mice. *J. Vet. Med. Sci.***2005,** *67*, 1005-1011.

Yeaman, M. R.; Yount, N. Y. Mechanisms of antimicrobial peptide action and resistance. *Pharmacol.Rev.***2003,** *55*, 27-55.

Zasloff, M. Magainins, a class of antimicrobial peptides from Xenopus skin: isolation, characterization of two active forms, and partial cDNA sequence of a precursor. *Proc. Natl. Acad. Sci. U S A.***1987,** *84*, 5449-5453.

Zeitler, B.; Herrera Diaz, A.; Dangel, A.; Thellmann, M.; Meyer, H.; Sattler, M.; Lindermayr, C. De-novo design of antimicrobial peptides for plant protection. *PLoS One.***2013,** *8*, e71687.

Zhang, L.; Benz, R.; Hancock, R. E. Influence of proline residues on the antibacterial and synergistic activities of alpha-helical peptides. *Biochemistry*.**1999,** *38*, 8102-8111.

Zwaal, R. F.; Schroit, A. J. Pathophysiologic implications of membrane phospholipid asymmetry in blood cells. *Blood*.**1997,** *89*, 1121-1132.