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UNRAVELING THE POTENTIAL OF ANTIMICROBIAL PEPTIDES DERIVED FROM BLACK SOLDIER FLY LARVAE, A REVIEW

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ABSTRACT

Antibiotic resistance is emerging in pathogens, which is highly alarming from a clinical perspective. In this regard, several types of natural compounds that could replace traditional antibiotics should be developed. *Hermetia illucens* or black soldier fly (BSF), is a saprophytic dipteran. Black soldier fly larvae (BSFL) feed and survive on decaying organic waste present in natural environments. As a result, they have to cope with a wide variety of pathogens, including bacteria, fungi, viruses, and others. As the first line of defense, they produce antimicrobial peptides (AMPs) as a result. These biological molecules have antibacterial, antiviral, antifungal, and even anticancer properties. They are made up of 22–50 amino acids. Large concentrations of AMPs can be detected in the hemolymph of BSFL. Following live or attenuated bacterial inoculation via micro-injections, AMP production in BSFL can be augmented. Additional techniques include treating BSFL with heat or acid or stabbing wounds. Dissolving the exterior lipid bilayer of pathogenic cells causes structural integrity to be disrupted and intracellular content to seep out of the cells, ultimately leading to the pathogens' death. They are unable to repair their damaged outer cellular lipid content and cytoplasmic leakage, which prevents them from developing resistance against BSFL-extracted AMPs. Therefore, AMPs generated from BSFL have the potential to replace traditional antibiotics without the risk of developing pathogenic resistance. This review elaborates on the production, classification, mechanism of action, and pharmaceutical value of AMPs derived from BSFLs.

KEYWORDS: Black soldier fly, bioactive molecule, hemolymph, antibiotics, innate immunity.

INTRODUCTION

Antibiotic resistance is a global health concern.[1] Antimicrobial Peptides (AMPs) are promising candidates to cope with it.[2] The mode of action of these AMPs shows an analogy with the traditional antibiotics. They have strong anti-pathogenic effects with no chance of developing resistant pathogens.[3] AMPs are evolutionarily conserved proteins synthesized in all living beings from prokaryotic bacteria to human beings.[4] As insects represent the highest number of species in the kingdom Animalia, they synthesize large amounts of AMPs as well. Diptera, Hymenoptera, Coleoptera, and Lepidoptera are the insect orders that are of great importance for synthesizing large amounts of AMPs.[5]

Hermetia illucens (Linnaeus, 1758) or black soldier fly (BSF) is one of the most appealing insects for extracting AMPs. The natural habitat of black soldier fly larvae (BSFL) is decaying organic waste, full of pathogens like bacteria, viruses, fungi, etc. This is the reason for the production of large concentrations of AMPs in them for protection against pathogens. They are the important component of BSFL innate immunity and provide the first line of defense during infections. They possess antibacterial, antifungal, antiviral, and antitumor effects.[6] The majority of AMPs in BSF comprise 22 to 50 amino acids.[7] They are synthesized in their fat body and hemocytes and released in hemolymph during infection.[8] This review summarizes the basics of AMPs in BSFL, their classification, their mechanism of action, and their medical importance.

Antimicrobial peptides of black soldier fly larvae

BSF, a non-pest dipteran, belonging to the Stratiomyidae family is a South American native insect. Nowadays, their ecological ranges have also been extended to tropical and temperate world regions, and are considered a cosmopolitan species.[9] The saprophytic black soldier fly larvae (BSFL) are around 20 mm long and 6 mm wide, whereas the adult is 13–20 mm long and resembles a black wasp.[10] Adult flies do not spread ailments because they lack mouthparts and stingers.[11] Numerous studies have shown that this is an insect of extreme importance since it is used in aquaculture, livestock, and poultry feed[12], as well as in waste management[13], agriculture[14], pharmaceutical[15], and the food sector.[16] They have a short life cycle, rapid growth, and

reproduction rate under optimal conditions. These attributes make them an important source of insect-based APMs to be used as antibiotics.[17]

The BSF genome synthesizes 50 AMPs, which is the largest number among all insect species.[18] BSFL produces AMPs only after activation of innate immunity by any pathogen infection. Under normal circumstances, the hemolymph of BSF contains minimal amounts of AMPs.[19] Injection of live or attenuated microbes to BSFL induces AMP expression. To date, different bacteria have been utilized for producing AMPs in BSFL. These include *Staphylococcus aureus*, *Lactobacillus casei*, *Escherichia coli*, *Bacillus subtilis*, *Xanthomonas oryzae* and *Micrococcus luteus*.[20] In addition, If BSFL is treated with thermal injury, large quantities of AMPs are produced due to the activation of innate immunity. Other methods that induce AMP expression in BSFL include thermal injury, stab wounds, or treatment with HCL or heat, etc.[21]

Classification of BSFL AMPs

There are five major categories of BSFL-derived AMPs. These include defensins, attacins, diptericins, cecropins, and knottin-like proteins, etc.[22] Both defensin and cecropins have structural similarities and these AMPs have been widely studied as compared to other AMPs to date. The structure of AMPs can be classified as α-helix, β-sheets, linear peptides with α- or β- elements, or a mix of both α- or β-structures.[23]

Cecropins

In 1974, cecropia AMPs were first isolated from the hemolymph of pupae of *Samia cynthia* or giant silk moth. Then in 1980, these were also detected from the hemolymph of pupae of *Hyalophora cecropia* or Cecropia moth. After the discovery of these AMPs in cecropia moth, insects gained attention for AMP isolation. These represent the most abundant class of insect AMPs.[24] They consist of 35 to 37 amino acids and possess an α-helical structure with no cysteine amino acid. In the BSFL genome, 7 genes encode for cecropins. Their mode of action involves lysis of bacterial cell membranes and reduction of uptake of proline. To date, they have been isolated from various insects, the adrenal glands of cattle, and the intestines of pork.[25]

These cationic AMPs are active against gram-positive as well as gram-negative bacteria. The gram-positive bacteria include Bacillus and Staphylococcus species. However, gram-negative bacteria include *Salmonella typhimurium*, *E. coli*, *Pseudomonas aeruginosa*, and *Klebsiella pneumonia*. They also have antifungal action against *Candida albicans*. The cecropin-like peptide 1 (CLP1) is 50 times more effective than against Ampicillin.[26]

The two cecropins i.e. Hill-Cec1 and Hill-Cec10 show strong antibacterial effects against *P. aeruginosa* strains, which are considered as the primary pathogens causing nosocomial infections. These cecropin AMPs can inactivate *P. aeruginosa* within 30 minutes. These have a fast mechanism of action of disrupting cell membranes, are less cytotoxic, highly active. Due to the strong antibacterial activity of these AMPs against *P. aeruginosa*, these can be utilized as a promising antipseudomonal drug for treating infections of the lungs or skin.[27]

Defensins

In BSFL, these are expressed by hemocytes or epithelial cells. These are the most common class of insect AMPs and are found to be present in various plants, animals, fungi, and insects. These cationic AMPs comprise 34 to 43 amino acids.[28] Their cationic nature is mainly due to cysteine, arginine, and lysine amino acids. However, cysteine is dominant in these antimicrobial proteins. These contain three disulfide bridges with 3 to 4 kDa molecular weight.[29] Their mode of action is similar to that of attacins. It involves the induction of necrosis in bacterial cell membranes (especially gram-positive), resulting in membrane pores and ultimately resulting in bacterial cell death due to the leakage of inside content through these pores.[30]

To date, 26 genes have been reported in BSFL encoding for defensins. These are effective against bacteria, fungi, protozoa, and yeast.[31] Other functions involve antifungal effects, wound healing, canine coat color expression, immunological regulation through interaction with melanocortin receptors, and maturation of mammalian sperm.[32] Hidefensin-1 is one of the most common and widely studied defensin AMP, with inhibitory action against *S. pneumoniae* and *E. coli*.[33]

Attacins

These were reported for the first time in a giant silk moth (*Hyalophora cecropia*). These are glycine-rich (14-22%) and other amino acids include aspartate, phenylalanine, alanine, and threonine.[34] In BSFL, 6 genes encode for attacins. They attach to lipopolysaccharides, part of the gram-negative bacterial outer membrane. After attachment, they inhibit the synthesis of the outer membrane of gram-negative bacteria, facilitating lysozymes and cecropins entry to the inside of bacterial cells. In addition, they protect against fungi and tumor cells.[35]

Diptericins

These glycine-rich AMPs were first identified in the blowfly (*Phormia terranova*). Their antipathogenic characteristics are more active against gram-negative bacteria. BSFL genome carries 10 AMPs encoding genes. The largest diptericin peptide in BSFL is reported to be Hiditericin-1. Their mode of action is not fully understood yet.[36]

Knottin-like proteins

These are disulfide-rich AMPs with an inhibitor cysteine knot and three interlinked disulfide bridges. These have

insecticidal, cytotoxic, and anti-protease activity. In BSFL, 4 genes are associated with synthesizing these peptides. They have been isolated from several insects, spiders, and cone snails.[37]

Mechanism of Action of Antimicrobial Peptides

AMPs target lipids of bacterial plasma membranes. As cationic, AMPs attach with anionic components of bacterial cells (Lipopolysaccharides, lipoteichoic acids, and teichoic acids). Eventually, they damage the bacterial cells' integrity by forming transmembrane pores.[38] These pores cause the leakage of cellular content to the outside environment. In gram-positive bacteria, AMPs attack the peptidoglycan layer. However, in the case of gram-negative bacteria, they target phosphate groups and phospholipids of their lipopolysaccharides (LPS).[39] Additionally, when LPS is released into the intercellular space of host cells, it binds to toll receptors, activating the immune system and ultimately causing sepsis.[40]

This bacterial membrane lysis involves 4 different mechanisms, 1) toroidal pore model, 2) carpet-like model, 3) barrel-stave model, and 4) unstructured ring pores.[41] In the toroidal pore model, pores are formed as AMPs damage the lipid bilayer of the bacterial membrane, resulting in bacterial cytoplasmic leakage.[42] The carpet-like model is associated with higher numbers of AMPs, which lyse the bacterial plasma membrane in a carpet-like mode. Cecropin AMPs exhibit this mechanism against pathogens. For example, large amounts of cecropins accumulate at the outer lipid bacterial membrane and form a carpet-like structure. This carpet-like structure acts like a detergent and results in the dissolving of the lipid bilayer, ultimately causing bacterial cell death.[43]

In the barrel stave model, AMPs attach with cell envelop, penetrate to the hydrophobic lipid bilayer, and result in intracellular content leakage of pathogenic cells. Amphotericin B and Cerototoxins AMPs show this mechanism of action.[44] However, the unstructured ring pore model involves the formation of transmembrane pores in the form of rings. In this mechanism, AMPs arrange in the form of rings at different angles attach to the pathogen's outer membrane and ultimately cause cell death.[45] Other than membranolytic effects, AMPs impair the intracellular metabolism of pathogenic cells. Pyrrhocoricin and drosocin AMPs damage the chaperone, which ultimately results in the failure of protein folding in pathogenic cells.[46]

Medical value of BSFL-derived AMPs

The peptides have the potential to be utilized in the pharmaceutical and biological domains, providing a novel approach to address the problem of antibiotic resistance. AMPs are specific in action, as they don't cause damage to normal eukaryotic host cells due to the presence of sphingomyelin and zwitterionic phosphatidylcholine on their neutral cell surfaces.[47] The antimicrobial evaluation of most of the BSF-derived AMPs has not yet been completely explored, but many studies demonstrated that they have promising effects against a wide range of infections.[48]

BSFL-extracted AMPs has the potential as an insect-based antibiotic, as the hemolymph extracted from BSFL, which were immunized with *Lactobacillus casei* provide good results against *Shigella dysenteriae* and *Klebsiella pneumoniae* in vitro.[49] Another study presented that the BSFL extract exhibits a strong antimicrobial action against methicillin-resistant bacteria, such as *Staphylococcus aureus* and *Pseudomonas aeruginosa*. Hence, it can be a potent antibiotic agent for antibiotic-resistant bacterial strains.[50] The methanolic extract of hemolymph obtained from mature BSFL, also shows strong antibacterial effects against *Micrococcus flavus, M. luteus, S. aureus, Klebsiella pneumoniae, Shigella sonnei, Neisseria gonorrhoeae, Salmonella typhimurium, P. aeruginosa*, and *E. coli*.[51]

Hidiptericin-1 and Hidefensin-1 AMPs provide pathogenicity against *Escherichia coli* and *Streptococcus pneumonia*[52], while Hill-Cec1 and Hill-Cec10 AMPs are toxic against *Pseudomonas aeruginosa and Klebsiella pneumonia.*[53] Three AMPs (Hidefensin-1, Hidiptericin-1, and HiCG13551) encoded by the BSFL genome enhance resistance against entomopathogenic infections in silkworms. Hence, BSFL-derived AMPs have a huge potential for controlling silkworm fungal infections.[54]

Recently, an extract of AMP named defensin-like peptide 4 (DLP4) was isolated from the hemolymph of BSF larvae. Methicillin-resistant Staphylococcus aureus (MRSA)-infected mice treated with DLP4 at doses ranging from 3 mg/kg to 7.5 mg/kg had an 80–100% survival rate. This discovery proved that DLP4 is a novel potential antimicrobial peptide (AMP) with promise against infections caused by MRSA. When added to feed, protein derived from the BSF considerably lowers the incidence of diarrhea in breeding animals and enhances their growth performance.[55] For many years, BSF AMPs have been the focus of intense research. It is anticipated that these compounds would be able to partially or completely prevent the horizontal transfer of resistance genes in the environment and replace antibiotics in poultry feed.[56]

Defensin-like peptides 2 and 4 (DLP2 and DLP4) lower the bacterial burden in the kidneys and spleen by over 95%, decrease proinflammatory cytokine levels in the blood, enhance anti-inflammatory cytokine levels, and heal lung and splenic injuries. DLP2 and DLP4 isolated from *Hermetia illucens* appear to be good options for treating staphylococcal infections. As a result, studies on AMPs from *Hermetia illucens* may be crucial in the field of biomedicine.[57] After feeding different insect meals including giant mealworm, yellow mealworm, house fly

larvae, etc. to diarrhea-affected piglets, the symptoms were decreased.[58]

BSFL were inoculated with attenuated *E. coli* during their late larval stage and after some hours, hemolymph was collected from them. This BSFL-extracted hemolymph was evaluated against *E. coli* and MRSA. The results showed the greatest antimicrobial activity in vitro. It was concluded that, if BSFL are immunized with live or attenuated bacteria during their late instar stage, it will lead to better production or extraction of anti-pathogenic AMPs from BSFL.[59] BSFL is also capable of being utilized for the synthesis of anti-Helicobacter pylori antibiotic drugs. The extraction of anti-H. pylori AMPs from BSFL are enhanced about 3 to 5 times if they are pre-immunized with E. coli.[60]

CONCLUSION

BSF is an insect of great medicinal, nutritional, and environmental importance. The most important bio-active molecules, i.e. AMPs protect BSFL against various pathogens. These AMPs carry the potential to replace traditional antibiotics. There is no possibility of pathogens to develop resistance against them. These can be easily extracted through the hemolymph of BSFL with the help of micro-injection. Several studies have concluded that BSFL-based AMPs have a broad spectrum of anti-pathogenic effects both in vitro and in vivo studies. Further research is needed to better understand their mode of action, more effective methods of stimulating their production in BSFL, the cascade of reactions that result in their production, more efficient ways of their extraction through BSFL-hemolymph, and identification of novel AMPs from BSFL.

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