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ANTIMICROBIAL PEPTIDES: PRINCIPAL DEFENSE CONSTITUENTS OF SILK WORMS - A REVIEW

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ABSTRACT

Insect has a highly developed innate immune system consisting of cellular and humoral responses. Antimicrobial peptides (AMPs) are the important components of the insect's humoral defense system and were first purified from the giant silk moth, *Hyalophora cecropia* L (Boman et al., 1980). For a variety of infections, they serve as the initial line of defense. AMPS are low molecular weight peptides and generally cationic in nature. Off late, the resistance of the pathogenic microbes towards established antibiotics have become a serious threat to global health. Over the years, an array of AMPs from natural sources has served as potent candidate against various infective agents viz. bacteria, fungi and viruses. These insect peptides not only exhibit antibacterial action by rupturing the microbial membrane but also prevent the development of drug resistance by microbes. Studies have shown AMPS to have synergistic effects with traditional antibiotics, providing an opportunity for combined therapy. Defensin, cecropins, drosocin, attacins, diptericins, ponericins, metchnikowins and melittin are a few classes of AMPs from insects that are currently isolated. However, the possibility of discovering new AMPs cannot be fully ruled out. Currently, 33 peptides are undergoing clinical trials, and 43% of the 77 AMPs are still in the preclinical stage (Makwana et al., 2023). Daptomycin and ovitavancin are two antibacterial lipopeptides that Food and Drug Administration has recently approved that are used for skin infection caused by bacteria. The present paper is an attempt to provide a comprehensive insight on antimicrobial peptides isolated from silk worm with reference to their structures, functions and possible mechanism of action.

Key words: Antimicrobial peptides, silk worm, *Philosamia ricini*, insects' immunity, cecropin, lebocin, moricin, attacin, defensin, gloverin, enbocin, isolation, characterization, microbes, multiple drug resistance

Silk worm belonging to the class Lepidoptera are one of the significant economic insects employed in molecular research (Goldsmith et al., 2005). Eri silk worm were known to be the earliest domesticated economic insects (Meng et al., 2017). They produce large offspring within a brief period of time, with larval stages lasting about 25-30 days (Fig. 1) and make silk which is a high-quality textile (Li et al., 2019). Sericulture is one of the major sources of income for many farmers in rural areas of India, China and Brazil (Wan et al., 2012). Besides the silk production, many immunological studies have been carried out on the bioactive properties of AMPs that seem to exhibit different mechanism with lesser side effects. Silk worms are being used as research model to study and understand the insect immunity and how better it can be implemented on human immune responses (Ravindar et al., 2015; Buhroo et al., 2018). One of the biggest risks to global public health is antimicrobial resistance. Antibiotic abuse in human and animal healthcare, particularly in the majority of developing countries, has caused threat in recent decades (Huan et al., 2020; Manniello et al., 2021; Ramzah et al., 2023). Scientists have predicted that antibiotic-resistance disease have the ability to kill millions of people per year and could cost the downfall of economy more than $100 trillion (Makwana et al., 2023). Development of new classes of antimicrobial drugs has slowed down over the past 35 years, which makes this undertaking much more challenging. As a result, there is an urgency of production and identifications of new antibiotic classes with therapeutics potential for treating variety of infectious disease in both humans and animals (Makwana et al., 2023).

Recently, screening of natural compounds with a wide range of chemical variations as significant and dependable sources of novel therapies is being pursued with interest (Uddin et al., 2021). One such class of naturally occurring substances is the AMPs, which are crucial elements of the innate immune system and are produced by variety of organisms in response to pathogenic stimuli (Dini et al., 2022).

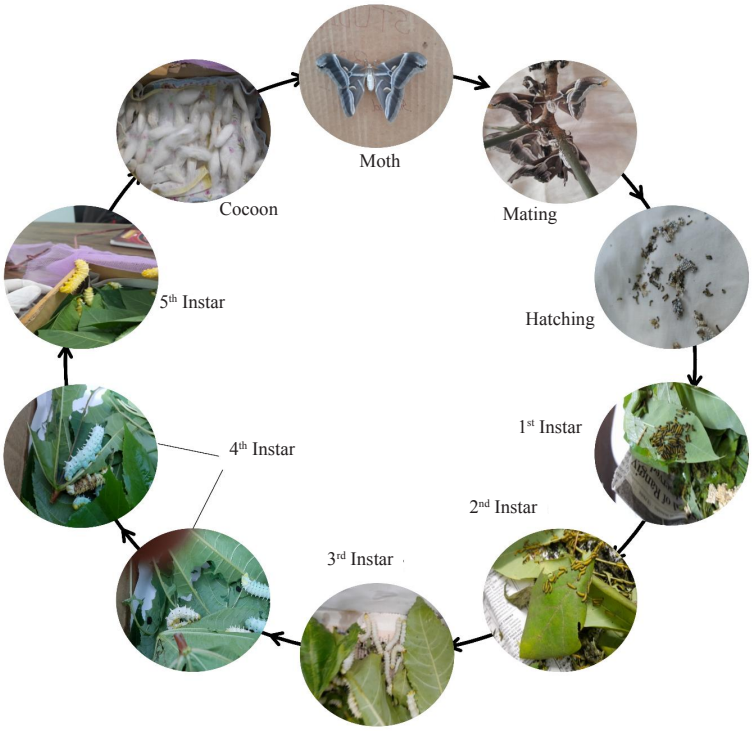

Fig. 1. Life cycle of *Philosamia ricini* L

Several established AMPs such as moricin, cecropin, gloverins, attacin etc. were reported to be found in insects, mammals, reptiles and plants. The silkworm has a number of benefits over other insects in the field of life science, including low breeding costs, huge offspring sizes, rapid generation time, and distinct genetic backgrounds (Meng et al., 2017). The discovery of gramicidin from a *Bacillus* bacterium strain in 1939 by Rene Dubos opens a way to isolate and characterized numerous AMPs (Huan et al., 2020; Moretta et al., 2021). AMPs from insect can inhibit Multiple drug resistance (MDR) bacteria such as *Escherichia coli, Klebsiella poneumoniae, Strptococcus sanguinus and Staphylococcus aureus*. A small number of AMPs from insects also displayed antiviral efficacy against the herpes simplex virus type 1 and the human influenzaa A and B viruses. Numerous fungal strains, including *Aspergillus* species, *Bptrytis* sp, *Cryptococcus* sp. and *Fusarium* sp. have also been documented to be vulnerable to AMPS from insects (Manniello et al., 2021). In antimicrobial peptide database nearly 3500 AMPs were registered from various taxa (Mahlapuu et al., 2020). Seven AMPs have been approved for use by the U.S Food and Drug Administration and are marketed as of a research report from 2020 (Chen and Lu, 2020). In addition to medicine related fields, AMPs are also reported to use in industries, including food processing, animal husbandry, aquaculture and agriculture (Erdem and Kesmen, 2022).

A. Insects and immune system

Studies reveal the rapid advancement in the research of human toxicology and pathology using the silkworm as a model animal. Several studies observed that silkworms are extremely susceptible to pesticides, antibiotics, pathogenic fungus and human pathogenic bacteria. There are established silkworm models for bacteria, fungal, viral, and natural immunological activation (Kaito and Sckimizu, 2007; Ishii et al., 2015a). So, the current Research area of interest has been using the silkworm as a model organism to study human malignancy, degenerative diseases and metabolic diseases has been gaining momentum (Meng et al., 2017).

An insect lacks adaptive immunity, yet they have an efficient and sophisticated innate immune system to distinguish and obliterate the powerful microbes (Cheng et al., 2008; Ravi et al., 2011). Structural barriers such as epidermis, midgut peritrophic membrane and tracheal respiratory organs along with haemocoel acts as the first line of defense against microbes (Tanaka et al., 2008). Their innate immune system comprises of cellular and humoral defense mechanism (Buhroo et al., 2018). In cellular immunity of insects, direct interactions between circulating hemocytes and microbes through phagocytosis, nodulation and encapsulation takes place (Rizki and Rikzi, 1984; Dularay and Lackie,

1985; Ratcliffe et al., 1985; Gupta, 1986; Boman and Hultmark, 1987; Gupta, 1991). Hemocytes are classified into five types based on their morphology: prohemocytes (Pr), granulocytes (Gr), plasmatocytes (Pl), spehrulocytes (Sp) and oenocytes (Oe) (Strand, 2008; Tan et al., 2013; Ribeiro and Brehelin, 2006; Lavine and Strand, 2002). In insects, prohemocytes are largely found in hematopoietic organs (HPOs) and have the ability to differentiate into other types of hemocytes (Minakhina and Steward, 2010). Plasmatocytes and granulocytes recognize the foreign substances and activates its immune responses by the process of phagocytosis (Lavine and Strand, 2002; Dubovskiy et al., 2016). Oenocytes are rich in prophenoloxidases (PPO) and are mostly engagaed in melanization mechanism, while spherulocytes functions are still to be discovered (Makwana et al., 2023). In humoral immunity, certain peptides known as antimicrobial peptides are produced in insect hemolymph upon microbial infection (Bulet et al., 1999). The fat body and occasionally, other immunocompetent tissues such as epithelium, midgut epithelium, and hemocytes are the key sites where the humoral immune responses is initiated (Pradeep et al., 2013; Jayaram et al., 2014; Lekha et al., 2014; Lekha et al., 2015; Makwana et al., 2021). This immunity involves the activation of signaling pathways like Toll, Immunodeficiency (Imd), and Janus kinase/signal transducers and activators of transcription (JAK/STAT) pathways in immune-responsive tissue and cells of host to create AMPs and other effector molecules (Makwana et al., 2023).

B. Antimicrobial peptides

Antimicrobial peptides are present in almost all organisms from vertebrates to invertebrates (Mastore et al., 2021). It was first purified from the immunized pupae of a giant silk moth, *Hyalophora cecropia* by Boman's group (Hultmark et al., 1980; Steiner et al., 1981). AMPs are low molecular weight proteins, which are heat stable, typically cationic and often less than 100 amino acid residues that are encoded by genes and synthesized by ribosomes having broad range of activities against the invading microbes (Ganz, 2003; Wang and Lai, 2010; Buhroo et al., 2018). Their deter-genic properties have the ability to disrupt the cell membranes of the intruding microbes and enzymatically attacks bacteria by hydrolyzing their cell wall which is made up of peptidoglycan (Dunn, 1986; Russell and Dunn, 1996). AMPs not only inhibit the bacterial growth, instead some of them cease the growth by interfering with protein synthesis, nucleic acid synthesis, cell division, or protease activity (Vanzolini et al., 2022). Insect fat bodies are responsible for production of AMPs (Brey et al., 1998; Hoffmann et al., 1999; Ganz, 2003; Schmid-Hempel, 2005).

According to different studies, AMPs are biologically active peptides vital for humoral immune response of insects against micro-organisms (Imura et al., 2007; Lemaitre and Hoffmann, 2007; Song et al., 2015). Not only in insects, many of the AMPs been discovered from other species having antibacterial, antifungal and antiviral activities (Wu et al., 2018). Some peptides of AMPs even have anticancer properties (Jin and Winberg, 2018). AMPs are active towards gram-positive and gram-negative bacteria (Tanaka et al., 2008). Additionally, they are also noted to be effective against fungi (Noorwala et al., 1994) and protozoa (Aley et al., 1994) showing minimal inhibitory concentrations (Hancock and Lehrer, 1998). Owing to its broad range of activity, lesser toxicity and low resistance against target cells, they are considered as probable candidates for the forthcoming drugs (Buhroo et al., 2018).

Numerous antibacterial proteins have so far been identified from several insect species (Cociancich et al., 1994a), and they can be divided into five main categories (Hultmark et al., 1980; Hultmark, 1993). They are Cecropins, insect defensins, attacin resembling proteins which are rich in glycine, proline-rich peptides and lysozymes. In immunized hemolymph of *B. mori*, peptides viz. cecropins, lysozyme and proline-rich proteins have been reported (Chadwick and Aston, 1991; Hara and Yamakawa, 1995). Seven different kinds of AMPs (cecropins, drosocin, attacins, *dipteracins*, defensin, drosomycin and metchnikowins) have been isolated, identified and characterized from *Drosophila* (Bulet et al., 1999; Irving et al., 2001; Hetru et al., 2003). Defensins, cecropins, proline-rich peptides and attacins are commonly found in insects, while gloverins and moricins are reported only in Lepidopteran insects (Yi et al., 2014). Fruit flies and mosquitoes belonging to the order Diptera produces AMPs such as defesin, attacins, cecropins, lebocins, gloverins, moricin, drosomycin and drosocin while only few have been identified and isolated from Lepidopteran species such as *B. mori, Antheraea mylitta, A. assamensis, A. pernyi* and *Manduca sexta* (Hara and Yamakawa. 1995; Dutta et al., 2016; Souhail et al., 2016; Nayak et al., 2017; Zhao et al., 2018).

C. Lepidopteran antimicrobial peptides

1. Cecropins

Cecropins are the cationic polypeptides first isolated from the hemolymph of a giant silk moth, *H. cecropia* (Steiner et al., 1981; Hultmark et al., 1982). In insects three kinds of cecropins are present, i.e., A, B and D which lacks strongly basic cysteine with a flexible glycine-proline link connecting the strong basic N-terminal to the neutral C-terminal with a length of 35-37 residue (Moore et al., 1996). Cecropins have a wide-spectrum of antimicrobial activities against different microbes and are widely studied (Bulet et al., 2004). According to previous studies, 30 cecropins were identified from *Papilionoidea* species (Wang et al., 2021). These peptides were also identified from silkworms *A. pyreni* and *B. mori* and many have been isolated from lepidopteran and dipteran insects (Buhroo et al., 2018). Cecropins are highly effective against bacteria irrespective of their types and to some extent it acts against some fungi (Buhroo et al., 2018). Bactericidin, lepidopteran and sarcotoxin are few other names of insect cecropin (Ouyang et al., 2015). Structurally related peptides of cecropin are shown in Table 1. Three distinct forms of cecropin were detected in insects: cecropin A (37 amino acids), cecropin B (35 amino acids) and cecropin D (37 amino acids). Genomic sequence of the silkworm revealed a total of 11 cecropin genes, which have been divided into 5 sub-types: A constituting Bmcec A1 (2 genes), B (6 genes), C (1 gene), D (1 gene) and E (1 gene) (Cheng et al., 2006; Yang et al., 2011; Nesa et al., 2020).

a) Cecropin A: Cecropin A shows the stable helical structure (Fu et al., 2004). The regulatory motifs of Cec-A genes have been discovered since the 1990s after the genes were cloned (Yamano et al., 1998; Yamakawa and Tanaka. 1999). Antibacterial mechanism of cec-A was unknown, however there is strong evidence that it works by targeting the cell membrane (Silvestro and Axelsen, 2000). Reactive Oxygen Species (ROS) are created when cec-A dramatically lowers NADPH and glutathione levels, which further increases oxidative stress (Durell et al., 1992; Yun and Lee, 2016). In silkworm larvae, Cec-A exhibits potential efficacy against the fungus, *Beauveria bassiana* (Lu et al., 2016).

b) Cecropin B: It is a linear-cationic peptide consisting of 35 amino acid that occurs naturally with the highest level of antibacterial activity among the cecropin family (Srisailam et al., 2000). Cecropin B significantly decreased the plasma endotoxin and *E. coli* load mortality in a rat model of septic shock stages (Giacometti et al., 2001). The nematode worm *Brugia pahongi's* adult female motility were reduces by Cec B, while in *Aedes aegypti* adult female, cec-B significantly reduces the quantity of larval development (Chalk et al., 1995). Additionally, cecropin B exhibits antifungal properties against *Candida albicans* (Andra et al., 2001) and it has a wide range of activity against porcine bacterial infections (Hu et al., 2013). Cecropin B is a potent antibacterial compound that has been expressed using transgenic technology in the cocoon of silkworm (Liu et al., 2015). Similar to this, a transgenic breed of silkworm can over-expressed Cecropin B1 to produce silk with universal antibacterial action (Saviane et al., 2018). The confinement of *Xanthomonas oryzae pv. oryzye* infections were aided by transgenic expressions of silkworm cecropin B in rice (Li et al., 2019).

c) Cecropin C: The hemolymph of *H. cecropia* contains trace amounts of cecropin C that were formed by degradation of cec A. Antibacterial properties of cec C has not been widely documented (Hultmark et al., 1982).

d) Cecropin D: Cecropin D was isolated from the bacteria infected, *H. cecropia* that exhibits similarities

Table 1. Lists of amino acid sequences of cecropin A, B, B1, B3, C, D and P1. (Wu, Patocka and Kuca, 2018)

Antimicrobial peptides	Amino acid sequence	References
Cecropin A	GGLKKLGKKLEGVGKRVFKASEKALPVAVGIKALG-NH2	Steiner et al., 1981
Cecropin B	KWKVFKKIEKMGRNIRNGIVKAGPAIAVLGEAKAL-NH2	Steiner et al., 1981
Cecropin B1	KWKVFKKIEKMGRNIRNGIVKAGPKWKVFKKIEK-NH2	Srisailam et al., 2001
Cecropin B3	AIAVLGEAKALMGRNIRNGIVKAGPAIAVLGEAKAL-NH2	Srisailam et al., 2001
Cecropin C	GWLKKLGKRIERIGQHTRDATIQGLGIAQQAANVAATAR-NH2	Hultmark et al., 1982
Cecropin D	WNPFKELEKVGQRVRDAVISAGPAVATVAQATALAK-NH2	Hultmark et al., 1982
Cecropin P1	SWLSKTAKKLENSAKKRISEGIAIAIQGGPR-NH2	Pillai et al., 2005

to cecropin A and cecropin B (Hultmark et al., 1982). Cecropin D were discovered later than Cecropin A or Cecropin B following bacterial infection in the hemolymph of an insects (Gudmundsson et al., 1991). Successful expression of recombinant cecropin D in *Pichia pastoris* revealed antibacterial efficacy against both Gram-positive and Gram-negative bacteria (Guo et al., 2012). Due to active phosphorylation, cecropin D's C-terminal lysine residue have more antibacterial action (Park et al., 2013). Additionally, in vitro replication and infection of the porcine reproductive and respiratory syndrome virus (PRRSV) were inhibited by cecropin D (Liu et al., 2015).

e) Cecropin E: Cecropin E were reported from the lepidopteran insects with 35 amino acid residues. Both gram-positive and gram-negative bacteria are found to be susceptible to cec E (Hultmark et al., 1982).

Amidation at the C-terminus plays an important role in enhancing the antimicrobial property of cecropin and helps the cecropins- liposomes intereaction (Naikajima et al., 1987). The cecropin-derived substances Sarcotoxin-1 (Okada and Natori, 1985), Papiliocin (Kim et al., 2010), Stomoxyn (Boulanger et al., 2002), Hinnavin (Yoe et al., 2006), SB-37, and Shiva (Jaynes et al., 1988) are active against the parasites like *Plasmodium* and *Trypanosoma* (Arrowood et al., 1991; Barr et al., 1995; Boisbouvier et al., 1998). SB-37 and Shiva also has the ability to inhibit the development of malignant cells and the HIV-1 virus (Chen et al., 1997; Suttmann et al., 2008).

2. Defensin

Defensin, a family of peptides isolated from the hemolymph of immunized flesh fly, *Sacophoga peregrine* (Matsuyama and Natori, 1988). Another insect defensin were also isolated from the hemolymph of the dipteran insect, *Phromia terranovae* (Lambert et al., 1989). They are cationic in nature having 4 kDa of peptides (Buhroo et al., 2018) and comprises of six conserved cysteine (crucial characteristics of defensins) molecules as Sapecins (Matsuyama and Naturi, 1988) which forms the intra-molecular disulphide bonds (Xiao et al., 2004). Phormicins, royalcins, sapecins, and spodoptericis are some of the antibacterial defensins that have been isolated and characterized from the order Lepidoptera (Fujiwara et al., 1990; Yamada and Natori, 1993). They are effective against gram-positive bacteria like *Micrococcus luteus, Aerococcus viridians, Bacillus megaterium, B. subtilis, B. thuringiensis, and S. aureus* (Yi et al., 2014). Besides that, few of antifungal defensin were too reported: drosomycin from *Drosophila*, heliomycin from tobacco budworm *Heliothis virescens* (Lamberty et al., 1999), *Galleria mellonella,* a caterpillar of the larger wax moth, produced gallerimycin, and *Pseudacanthotermes spiniger*, an isopteran, produced termicin. (Lamberty et al., 2001b), and Alo13 from the order Coleoptera, harlequin beetle *Acrocinus longimanus* (Barbault et al., 2003). Insect defensins were isolated and reported from several insect orders such as dipteran, hymenopteran, coleopteran, trichopteran, hemipteran and odonata (Hoffmann and Hetru, 1992; Cociancich et al., 1994 b).

The hemocytes, silk gland, head, and ovary of the larvae as well as the fat body of the pupae were all shown to be strongly transcribed with the gene BmDefensinA, which was discovered from the genome of *B. mori*. This gene Bmdefensin regulates the immune challenges of the insect and play an important role in both immunity and metamorphosis of insects (Wen et al., 2009). A new defensin-like gene called Spodoptericin is modestly up-regulated by bacteria and fungus. It was observed that the sporoptericin gene lacked any sequence pattern that was clearly homologous to the cis regulatory element controlling antimicrobial peptide genes (Volkoff et al., 2003). When characterized BmDefA gene, it was reported that the gene contains 5' upstream regulatory region having three conserved regulatory sequence of cis elements i.e., NF-kβ binding site, IL-6 responsive element and GATA motif. This gene BmDefA when translate encode large peptides with N-terminal signal peptide (22 amino acid), a propeptide (34 amino acid) and a mature peptide (36 amino acid) with a molecular weight of 4 kDa. Mature BmDefA have the isoelectric point with a value of 4.2 leading to the discovery of novel anionic defensin (Buhroo et al., 2018; Nesa et al., 2020). BmDefensinB was also identified from the fat body of silkworm *B. mori* which acts as the homolog of insect defensin, with three NF-B binding sites and other cis elements (KAIKOBLAST). The findings imply that BmDefensinA and BmDefensinB contribute to *B. mori's* immunity (Kaneko et al., 2008). This defensin has only 27% of similarity in amino acid when compared to BmDefA and get activated by bacteria such as *E. coli* and *B. subtilis* and also by *Beauveria bassiana*. When used against *Pseudomonas aeruginosa* MDR strains, rabbit neutrophils defensins exhibit strong bactericidal actions (Zhao et al., 2005). Toll and Imd pathway control the gene expression of BmDefB and plays an important role in regulation of immune reaction in both bacteria and fungi in the organism *B. mori* (Buhroo et al., 2018). Amino acid sequence of defensin is shown in Table 2.

Table 2. Comparision of the gene BmDefensinB and BmDefensinA from *B. mori (*Kaneko et al., 2008)

BmDefensinB	--ALPCAK----KS-CDSWCRRLDYPCGECVTKWKCSCNNWMQ DK	38
BmDefensinA	---IWCEFEEATETAI CQEHCLPKGYSYGI CVSN-TSCCI-----	36

3. Moricin

Moricin are highly basic 42-residue peptide that was first isolated from the *B. mori* immunized with *E. coli* and thus peptide actively suppressed the growth of *S. aurens* (Hara and Ymakawa, 1995). Moricin has only ever been discovered in Lepidopteran insects, and cDNAs encoding it have been found in *M. sexta* (Zhu et al., 2003), *Spodoptera litura* (Oizumi et al., 2005), *G. mellonella* (Brown et al., 2008), *Helicoverpa armigera* (Wang et al., 2010b) and other insects. The cDNA sequences of *S. exigua*, *Heliothis virescens*, and *Hyblaea puera* are also available in the NCBI database (Yi et al., 2014). According to Christensen et al. 1988; Gabay, 1994, the basicity is assumed to be the cause of the attachment of positively charged peptides to the negatively charged bacterial surface through the electrostatic interaction. Moricin's isoelectric point was determined to be 12.0, greater than cecropin's (pI=8.2-9.6). Secondly, an amphipathic α-helix structure has been observed present in moricin that are often in charge of expressing antibacterial action (Hara and Yamakawa, 1995). *B. mori* genome have 12 moricin-coding genes that can be divided into three subtypes, i.e., Bmmor (1 gene), moricin-like A (3 genes) and moricin-like B (8 genes) and above all, Bmmor gene exhibits highest antibacterial activity against bacteria (Yang et al., 2011). In an in vitro experiment, moricin from *B. mori* (Bmmor) was found to have antibacterial properties against *E. coli, B. bombysepticus, B. subtilis, B. thuringiensis, B. thuringiensis galleriae, Serratia marcescens, S. aureus, P. aeruginosa,* and *Ralstonia solaanacearum* at dosages of 0.625-1.25 mL/L (Rd). Bmmor, a member of the *B. mori* moricin family, has the highest antibacterial toxicity (p 0.01) and is followed by the moricin-like A subtype. The B subtype of moricin, in contrast, has no effect on the tested microorganisms (Yang et al., 2011).

Genes encode with mature moricin, contain a positively charged C-terminus and an amphipathic alpha helical N-terminus without any post-translational modification paving a way for synthesizing moricin chemically (Islam et al., 2016). The α-helical motif of moricin plays an important role in increasing the membrane permeability to destroy pathogen (Yi et al., 2014). Charged amino acids can be seen in the N-terminal half of moricin at intervals of three or four amino acid residues, indicating a structure common to antibacterial proteins with the amphipathic α-helix (Cociancich et al., 1994a; Kreil, 1994). Both gram-positive and gram-negative bacteria are sensitive to moricins; and the moricins that was isolated from *G. mellonella* shows high activity against filamentous fungi and yeasts (Hara and Yamakawa, 1995a; Brown et al., 2008; Dai et al., 2008). Moricin have a significant role in *B. mori's* self-defense against bacterial infection since it was produced in response to bacterial infection and exhibits substantial antibacterial activity (Hara and Yamakawa, 1995).

4. Attacins

The peptide attacin with a molecular weight of 20-23 kDa are glycine-rich protein (Wu et al., 2018) and were first isolated from *H. cecropia* pupae (Hultmark et al., 1983; Buhroo et al., 2018). Attacin are found in both acidic and basic form with an isoelectric point ranging from 5.7 to 8.3 (Nesa et al., 2020). Six isolated attacin AMP were classified into 2 groups: a basic group constituted by attacin A to D and an acidic group comprising of attacin E and F (Buonocore at al., 2021). Acidic attacin has a high contain of aspartic acid, arginine and isoleucine, whereas basic attacin has a high contain of lysine, glutamic and tryptophan (Islam et al., 2016). Amino sequence of attacins A-F is shown in Table 3. Attacin shows antibacterial activity against *E. coli, Stenotrophomonas maltophilia* and *Acinetobacter calcoaceticus* (Nesa et al., 2020). Attacin blocks the synthesis of outer membrane proteins of gram-negative bacteria which disturbed the cell wall of bacteria causing a chain or long chain formation (Carlsson et al., 1998; Nesa et al., 2020). Attacin are third antibacterial protein found in the humoral immune system of *H. cecropia* after cecropin and lysozyme (Kockum et al., 1984).

Table 3. Amino-terminal sequence of attacin A-F. Their sequence difference can be observed from the highlighted residue (Wu et al., 2018)

Attacin A-C	AGALTINSDGTSGAVVKVPI- NH2
Attacin D-F	DAHGALTLNSDGTSGAVVKVPFAGNDLNI- NH2

5. Gloverin

Gloverin is a basic antibacterial protein with a molecular weight of ~14 kDa isolated from the pupal haemolymph of giant silk moth, *H. gloveri* (Axen et al., 1997; Buhroo et al., 2018; Nesa et al., 2020). This peptide contains 18.5% of glycine rich amino acid residue and lacks cysteine residue (Axen et al., 1997). This peptide is active against gram-negative and gram-positive bacteria and shows a bit similarity to attacin peptides (Kaneko et al., 2007). Pro-gloverin feature a conserved RXXR motif at their N-terminal region, and gloverins are produced as pre-pro proteins (Xu et al., 2012). The N-terminal pro-regions (22–26 residues) are likely eliminated by furin-like enzymes before the synthesized of mature gloverins. However, recombinant *Trichoplusia ni* pro-gloverin is equally as effective against *E. coli* as mature gloverin from *H. gloveri* (Lundstorm et al., 2002), indicating that some pro-gloverins may not require the removal of pro-regions in order to be active (Yi et al., 2014). Gloverin was said to expressed only in lepidopteran insects, namely *Helicoverpa armigera* (Mackintosh et al., 1998a), *T. ni* (Lundstrom et al., 2002), *A. mylitta* (Gandhe et al., 2006), *M. sexta* (Abdel-latief and Hilker, 2008; Xu et al., 2012), *Diatrea saccharialis* (Kawaoka et al., 2008; Mrinal and Nagaraju, 2008). *E. coli* infected silkworm produce this peptide in the fat body of insects (Mrinal and Nagaraju, 2008) and detail study about their genome revealed that the silkworm produces 4 genes encoding Bmglv 1,2,3 and 4 (Yang et al., 2011). Among them, *B. mori* gloverin 1 is the ancestral gene and gloverin 2-4 are produce by the duplication of gloverin 1 gene (Mrinal and Nagaraju, 2008). In insect *B. mori*, Gloverin 1 were found to be expressed in the gonads of larval pupae of silkworm but not in the adults, while gloverin 2-4 are expressed only on adults' gonads of silkworm (Mrinal and Nagaraju, 2008). In *M. sexta,* this peptide is highly expressed in the testis of naive larvae (Xu et al., 2012). Detailed studies about gloverin peptide leads to the conclusion that these peptides not only have the antimicrobial activity but also plays an important role in the development and reproduction of insects (Yi et al., 2014).

6. Lebocin

Lebocin were initially discovered as proline-rich, O-glycosylated 32-residue peptides in the hemolymph of *E. coli* immunized silkworm *B. mori* (Hara and Yamakawa, 1995b). According to a complementary DNA clone for *B. mori* lebocin, the active 32-residue peptide is situated adjacent to the C-terminus of the precursor protein, which contains 179 residues (Choudhury et al., 1995). The amino acid residue (15-Thr) is a crucial component of antibacterial action (Islam et al., 2016). These peptide fundamental structure and antibacterial properties mostly match with those of abaecin (41% of same amino acid sequence) of honey bees that have 34-residue of the proline-rich peptide and devoid of O-glycosylated (Casteels et al., 1990).

Lebocin 1 and lebocin 2 are two distinct analogues of lebocin that share identical amino acid residue but differ in the length of their sugar chains on the threonine residues. According to reports, these antibacterial proteins with glycosylated threonine include various sugars, such as galactose, N-acetyl galactosaminein lebocin 1 and N-acetyl galactoseamine in lebocin 2. Amino acid sequence of lebocine 3 showed one amino acid replacement at 16-Leu and 15-Thr. Lebocin amino acid sequence shows similarity with other members of lebocin (Islam et al., 2016). Amino acid sequence of lebocin is shown in Table 4. In vitro research discovered that lebocin promotes lipid bilayer leaking underneath low ionic circumstances that could indicate active rupture of bacterial membrane (Hara and Yamakawa, 1995). Due to its low sensitivity and the requirement for a low ionic conductivity, however, its specific mechanism is still being studied. Lebocin 3 has an additive cecropin D, as their conjugation significantly boosts the cecropin D's antimicrobial effects on *B. mori* (Ponnuvel and Yamakawa, 2002).

7. Enbocin

Enbocin is a novel antimicrobial peptide having 59 amino acid residues identified from *B. mori* (Kaneko et al., 2007). In 1998, a silkworm cDNA fragment was cloned to screen the first gene expression named as enbocin. After performing an experiment, it was reported that the amino acid sequence of enbocin belongs to cecropin family (Kim et al., 1998). Although, their antibacterial properties differ from that of cecropin

Table 4. Amino acid Sequences of Lebocin (Nesa et al., 2020)

Lebocin 1	DLRFLYPRGKLPVPTPPPENPKPIYIDMGNRY
Lebocin 2	DLRFLYPRGKLPVPTPPPENPKPIYIDMGNRY
Lebpcin 3	DLRFLYPRGKLPVPTPPPENPKPIYIDMGNRY
Lebocin 4	DLRFLYPRGKLPVPTPPPENPKPITIDMGNRY

(Kim et al., 1998). This enbocin exhibits antibacterial activity against gram-positive and gram-negative bacteria. In silkworm, at least 2 enbocin genes are present (Kaneko et al., 2007). According to the analysis of induction mechanism, the expression of silkworm enbocins is induced by the bacteria *E. coli* and thereafter occurs mostly in the fat body (Li et al., 2019). Amino acid sequences were shown in Table 5.

CONCLUSIONS

Insect AMPs exhibit antibacterial action against a variety of microorganisms. Despite their small size, AMPs are becoming increasingly popular due to their incredible qualities and capabilities. Because they can generate more potent and stable peptides, smaller peptides of insect AMPs are regarded to be a superior candidate for medical applications. Silkworms have recently gained popularity in the field of life sciences for applications such as environmental safety monitoring and pharmaceutical pathogen screening. As the number of diseases resistant to more than one antibiotic increases, the efficacy of several medications falls. This motivates scientists to seek out new sources of bioactive chemicals that naturally destroy germs. Silkworm AMPs has focused mainly on antibacterial properties, severely limiting their use in other domains. A thorough examination of the role and application of silkworms in the manufacture of AMP is necessary. The silkworm model has been applied successfully in numerous domains of life science study, significantly improving scientific research in this area. Many hurdles remain, and the application of the silkworm model in many disciplines is still in its infancy due to a lack of evidence from clinical trials and adequate animal investigations.

Finally, boosting the use of silk worm models in scientific research will bring new insights into long-held problem-solving assumptions, helping both science and society greatly. This model organism offered a bright future to the researchers. The activity of silk worm AMPs determines their relevance for applications. Silk worm AMPs have numerous applications, including biomaterials, pharmaceuticals, food bio preservation, and disease prevention in plants and animals. According to medical professionals, silk worm AMPs are promising antibiotic candidates that can be transformed into powerful anticancer and antiparasitic medications. The discovery and classification of AMPs, an important effector of the innate immune system, are significant in demonstrating how AMP variations can be developed to combat the increased prevalence of multi-drug resistant infections as a viable alternative to traditional antibiotics. This study highlights silk worm as a potential source of antimicrobial peptides and opens up new channels for intervention and the creation of naturally occurring bioactive chemicals to combat antibiotic resistance.

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REFERENCES

Abdel-latief M, Hilker M. 2008. Innate immunity: eggs of *Manduca sexta* are able to respond to parasitism by *Trichogramma evanescens*. Insect Biochemistry and Molecular Biology 38(2): 136-145.

Aley S B, Zimmerman M, Hetsko M, Selsted M E, Gillin F D. 1994. Killing of *Giardia lamblia* by cryptdins and cationic neutrophil peptides. Infection and Immunity 62(12): 5397-403.

Andrä J, Berninghausen O, Leippe M. 2001. Cecropins, antibacterial peptides from insects and mammals, are potently fungicidal against *Candida albicans*. Medical Microbiology and Immunology 189: 169-173.

Al Souhail Q, Hiromasa Y, Rahnamaeian M, Giraldo M C, Takahashi D, Valent B, Vilcinskas A, Kanost M R. 2016. Characterization and regulation of expression of an antifungal peptide from hemolymph of an insect, *Manduca sexta*. Developmental and Comparative Immunology 61: 258-268.

Arrowood M J, Jaynes J M, Healey M C. 1991. In vitro activities of lytic peptides against the sporozoites of *Cryptosporidium parvum*. Antimicrobial Agents and Chemotheraphy 35(2): 224-227.

Axén A, Carlsson A, Engström A, Bennich H. 1997. Gloverin an antibacterial protein from the immune hemolymph of *Hyalophora* pupae. European Journal of Biochemistry 247(2): 614-619.

Barr S C, Rose D, Jaynes J M. 1995. Activity of lytic peptides against intracellular *Trypanosoma cruzi* amastigotes in vitro and parasitemias in mice. Journal of Parasitology 81(6): 974-978.

Barbault F, Landon C, Guenneugues M, Meyer J P, Schott V, Dimarcq J L, Vovelle F. 2003. Solution structure of Alo-3: a new knottin-type antifungal peptide from the insect *Acrocinus longimanus*. Biochemistry 42(49): 14434-14442.

Boisbouvier J, Prochnicka-Chalufour A, Nieto A R, Torres J A, Nanard N, Rodriguez M H, Possani D L, Delepierre M. 1998. Structural

Table 5. Amino acid sequence of Enbocin, Enbocin 2 and Enbocin 3 (Kaneko et al., 2007)

Enbocin	MNFTRIIFFLFVVVFATASGKPWNFKEIERAVARTRDAVISAGPA RTVAAATSVASG
Enbocin 2	MNFTRIIFFLFVVVFATASAKPWNFFKEIERAVARTRDAVISAGPAVATVGAAAAVASG
Enbocin 3	MNFTRIIFFLFVVVFATASAKPWNFFKEIERAVARTRDAVISAGPAVATVAAASAVASG

information on a cecropin-like synthetic peptide, Shiva-3 toxic to the sporogonic development of *Plasmodium berghei*. European Journal of Biochemistry 257(1): 263-273.

Boman H G, Hultmark D. 1987. Cell-free immunity in insects. Annual Review of Microbiology 41(1): 103-126.

Boulanger N, Brun R, Ehret-Sabatier L, Kunz C, Bulet P. 2002. Immunopeptides in the defense reactions of *Glossina morsitans* to bacterial and *Trypanosoma brucei brucei* infections. Insect Biochemistry and Molecular Biology 32(4): 369-375.

Brown S E, Howard A, Kasprzak A B, Gordon K H, East P D. 2008. The discovery and analysis of a diverged family of novel antifungal moricin-like peptides in the wax moth *Galleria mellonella.* Insect Biochemistry and Molecular Biology 38(2): 201-212.

Brey P T, Hultmark D. 1998. Molecular Mechanisms of Immune Responses in Insects. (Eds), Chapman & Hall, London/New York, 1998.

Bulet P, Hetru C, Dimarcq J L, Hoffmann D. 1999. Abtimicrobial peptides in insects; structure and function. Developmental and Comparative Immunology 23(4-5): 329-344.

Bulet P, Stöcklin R, Menin L. 2004. Anti-microbial peptides: from invertebrates to vertebrates. Immunological Review 198(1): 169–184.

Buhroo Z I, Bhat M A, Bali G K, Kamili A S, Ganai N A. 2018. Antimicrobial peptides from insects with special reference to silkworm *Bombyx mori* L.: A review. Journal of Entomology and Zoology Studies 6: 752-759.

Buonocore F, Fausto A M, Pelle G D, Roncevic T, Gerdol M, Picchietti S. 2021. Attacins: A Promising Class of Insect Antimicrobial Peptides. Antibiotics 10(2): 212.

Casteels P, Ampe C, Riviere L, Van Damme J, Elicone C, Fleming M, Jacobs F, Tempst P. 1990. Isolation and characterization of abaecin, a major antibacterial response peptide in the honeybee (*Apis mellifera).* European Journal of Biochemistry 187(2): 381-386.

Carlsson A, Nyström T, de Cock H, Bennich H. 1998. Attacin—An insect immune protein—Binds LPS and triggers the specific inhibition of bacterial outer-membrane protein synthesis. Microbiology 144(8): 2179-2188.

Chalk R, Townson H, Ham P J. 1995. *Brugia pahangi*: the effects of cecropins on microfilariae in vitro and in *Aedes aegypti*. Experimental Parasitology 80(3): 401–406.

Chadwick J S, Aston W P. 1991. Antibacterial immunity in Lepidoptera. In: Gupta AP, editor. Immunology of insects and other arthropods. Boca Raton: CRC Press; 1991, 347-370.

Cheng T, Zhao P, Liu C, Xu P, Gao Z, Xia Q, Xiang Z. 2006. Structures, regulatory regions, and inductive expression patterns of antimicrobial peptide genes in the silkworm *Bombyx mori.* Genomics 87(3): 356-365.

Cheng T C, Zhang Y L, Liu C, Xu P Z, Gao Z H, Xia Q Y, Xiang Z H. 2008. Identification and analysis of Toll-related genes in the domesticated silkworm, *Bombyx mori.* Developmental and Comparative Immunology 32(5): 464-475.

Chen H M, Wang W, Smith D, Chan S C. 1997. Effects of the anti-bacterial peptide cecropin B and its analogs, cecropins B-1 and B-2, on liposomes, bacteria, and cancer cells. Biochimica et Biophysica Acta (BBA)-General subjects 1336(2): 171-179.

Chen C H, Lu T K. 2020. Development and challenges of antimicrobial peptides for therapeutic applications. Antibiotics 9(1):24.

Chowdhury S, Taniai K, Hara S, Kadonookuda K, Kato Y, Yamamoto M, Xu J, Choi S K, Debnath N C, Choi H K, Miyanoshita A, Sugiyama M, Asaoka A, Yamakawa M. 1995. cDNA cloning and gene expression of lebocin, a novel member of antibacterial peptides from the silkworm, *Bombyx mori.* Biochemical and Biophysical Research Communications 214(1): 271-278.

Christensen B C, Fink J, Merrifield R B, Mauzerall D. 1988. Channel-forming properties of cecropins and related model compounds incorporated into planar lipid membranes. Proceedings of the National Academy of Science 85(14): 5072-5076.

Cociancich S, Bulet P, Hetru C, Hoffmann J A. 1994. The inducible antibacterial peptides of insects. Parasitol Today 10(4): 132-139.

Dai H, Rayaprolu S, Gong Y, Huang R, Prakash O, Jiang H. 2008. Solution structure, antibacterial activity, and expression profile of *Manduca sexta* moricin. Journal of Peptide Science: An Official European Peptide Society 14: 855-863.

Dini I, De Biasi M G, Mancusi A. 2022. An overview of the potentialities of antimicrobial peptides derived from natural sources. Antibiotics 11(11): 1483.

Dubovskiy I M, Kryukova N A, Glupov V V, Ratcliffe N A. 2016. Encapsulation and nodulation in insects. Invertebrate Survival Journal 13(1): 229-46.

Dularay B, Lackie A M. 1985. Haemocytic encapsulation and prophenoloxidase activation pathway in the locust *Schisocerca gregaria.* Forsk. Insects Biochemistry 15(6): 827-834.

Dunn P E. 1986. Biochemical aspects of insect immunology. Annual Review of Entomology 31(1): 321-339.

Durell S R, Raghunathan G, Guy H R. 1992. Modeling the ion channel structure of cecropin. Biophysical Journal 63(6): 1623–1631.

Dutta D, Kumar N, Willcox M D P. 2016. Antimicrobial activity of four cationic peptides immobilized to poly-hydroxyethylmethacrylate. Biofouling 32(4): 429-438.

Erdem Büyükkiraz, M.; Kesmen, Z. 2022. Antimicrobial peptides (AMPs): A promising class of antimicrobial compounds. J. Appl. Microbiol. 132: 1573-1596.

Fu H, Björstad A, Dahlgren C, Bylund J. 2004. A bactericidal cecropin-A peptide with a stabilized α-helical structure possess an increased killing capacity but no proinflammatory activity. Inflammation 28(6): 337-343.

Fujiwara S, Imai J, Fujiwara M, Yaeshima T, Kawashima T, Kobayashi K. 1990. A potent antibacterial protein in royal jelly. Purification and determination of the primary structure of royalisin. Journal of Biological Chemistry 265(19): 11333-11337.

Gabay J E. 1994. Ubiquitous Natural science. Science 264(5157): 373-374.

Gandhe A S, Arunkumar K P, John S H, Nagaraju J. 2006. Analysis of bacteria-challenged wild silkmoth, *Antheraea mylitta* (lepidoptera) transcriptome reveals potential immune genes. BMC Genomics **7**(1): 1-10.

Ganz T. 2003. Defensins: antimicrobial peptides of innate immunity. Nature reviews immunology 3(9): 710-720.

Giacometti A, Cirioni O, Ghiselli R, Viticchi C, Mocchegiani F, Riva A, Saba V, Scalise G. 2001. Effect of mono-dose intraperitoneal cecropins in experimental septic shock. Critical care medicine 29(9): 1666-1669.

Goldsmith M R, Shimada T, Abe H. 2005. The genetics and genomics of the silkworm, *Bombyx mori*. Annual review entomology 50: 71-100.

Gudmundsson G H, Lidholm D A, Asling B, Gan R, Boman H G. 1991. The cecropin locus. Cloning and expression of a gene cluster encoding three antibacterial peptides in *Hyalophora cecropia*. Journal of Biological Chemistry 266(18): 11510-11517.

Guo C, Huang Y, Zheng H, Tang L, He J, Xiao L, Liu D, Jiang H. 2012. Secretion and activity of antimicrobial peptide cecropin D expressed in *Pichia pastoris*. Experimental and therapeutic Medicine 4(6): 1063-1068.

Gupta A P. 1986. Hemocytic and Humorul Immunity in Arthropods. Wiley, New York, 1986

Gupta A P. 1991. Immunology of insects and other arthropods. CRC press.

Hancock R E, Lehrer R. 1998. Cationic peptides: a new source of antibiotics. Trends in Biotechnology 16(2): 82-88.

Hara S, Yamakawa M. 1995. Moricin, a novel type of antibacterial peptide isolated from the silkworm, *Bombyx mori* (*). Journal of Biological Chemistry 270(50): 29923-29927.

Hara S, Yamakawa M. 1995. A novel antibacterial peptide family isolated from the silkworm, *Bombyx mori.* Biochemical Journal 310(2): 651–656.

Hetru C, Troxler L, Hoffmann J A. 2003. *Drosophiola melanogaster* antimicrobial defense. Journal of Infectious Diseases 187(Supplement_2): 327–S343.

Hoffmann J A, Hetru C. 1992. Insect defensins: Inducible antibacterial peptides. Immunology today 13(10): 411-415.

Hoffmann J A, Kafatos C F, Jr Janeway A C, Ezekowitz B A R. 1999. Phylogenetic perspective in innate immunity. Science 284(5418): 1313-1318.

Huan Y, Kong Q, Mou H, Yi H. 2020. Antimicrobial peptides: classification, design, application and research progress in multiple fields. Frontiers in microbiology p. 2559.

Hultmark D, Steiner H, Rasmuson T, Boman H G. 1980. Insect immunity. Purification and properties of three inducible bactericidal proteins from hemolymph of immunized pupae of Hyalophora cecropia. European Journal of Biochemistry106(1): 7-16.

Hultmark D, Engström A, Bennich H, Kapur R, Boman H G. 1982. Insect immunity. Isolation and structure of cecropin D and four minor antibacterial components from *cecropia pupae.* European Journal of Biochemistry 127(1): 207-217.

Hultmark D, Engström A, Andersson K, Steiner H, Bennich H, Boman H G. 1983. Insect immunity. Attacins, a family of antibacterial proteins from *Hyalophora cecropia.* The EMBO Journal 2(4): 571-576.

Hultmark D. 1993. Immune reactions in Drosophila and other insects: a model for innate immunity. Trends in Genetics 9(5): 178-183.

Hu H, Wang C, Guo X, Li W, Wang Y, He Q. 2013. Broad activity against porcine bacterial pathogens displayed by two insect antimicrobial peptides moricin and cecropin B. Molecules and Cells 35: 106-114.

Imura Y, Nishida M, Matsuzaki K. 2007. Action mechanism of PEGylated magainin 2 analogue peptides. Biochimica et Biophysica Acta (BBA) – Biomembranes 1768(10): 2578-2585.

Irving P, Troxler L, Heuer T S, Belvin M, Kopczynski C, Reichhart J M, Hoffmann J A, Hetru C. 2001. A genome-wide analysis of immune responses in Drosophila. Immunology 98(26): 15119-15124.

Islam J S, Bezbaruah S and Kalita J. 2016. A review on antimicrobial peptides from *Bombyx mori* L and their application in plant and animal disease control. Journal of Advances in Biology & Biotechnology 9(3): 1-15.

Ishii K, Hamamoto H, Sekimizu K. 2015. Studies of host-pathogen interactions and immune-related drug development using the silkworm: interdisciplinary immunology, microbiology, and pharmacology studies. Drug discoveries and Therapeutics 9(4): 238-246.

Jaynes J M, Burton C A, Barr S B, Jeffers G W, Julian G R, White K L, Enright F M, Klei T R, Laine R A. 1998. In vitro cytocidal effect of novel lytic peptides on *Plasmodium falciparum* and *Trypanosoma cruzi*. The FASEB Journal 2(13): 2878-2883.

Jayaram A, Pradeep A N R, Awasthi A K, Murthy G N, Ponnuvel K M, Sasibhushan S, Rao G C. 2014. Coregulation of host–response genes in integument: switchover of gene expression correlation pattern and impaired immune responses induced by dipteran parasite infection in the silkworm, *Bombyx mori*. Journal of Applied Genetics 55: 209-221.

Jin G, Weinberg A. 2018. Human antimicrobial peptides and cancer. In Seminars in Cell and Developmental Biology 88: 156-162.

Kaito C, Sekimizu K. 2007. A silkworm model of pathogenic bacterial infection. Drug Discovery and Therapeutics 1(2): 89-93.

Kaneko Y, Furukawa S, Tanaka H, Yamakawa M. 2007. Expression of antimicrobial peptide genes encoding Enbocin and Gloverin isoforms in the silkworm, *Bombyx mori*. Bioscience, Biotechnology and Biochemistry 71(9): 2233-2241.

Kaneko Yoichi, Tanaka Hiromitsu, Ishibashi J, Wasaki T, Yamakawa M. 2008. Gene expression of a novel defensin antimicrobial peptide in the silkworm, *Bombyx mori*. Bioscience, Biotechnology and Biochemistry 72(9): 2353-2361.

Kawaoka S, Katsuma S, Daimon T, Isono R, Omuro N, Mita K, Shimada T. 2008. Functional analysis of four Gloverin-like genes in the silkworm, *Bombyx mori.* Archives of Insect Biochemistry and Physiology: Published in collaboration with Entomological society of America 67(2): 87-96.

Kim S H, Park B S, Yun F Y, Je Y H, Woo S D, Kang S W, Kim K Y, Kang S K. 1998. Cloning and expression of a novel gene encoding a new antibacterial peptide from silkworm, *Bombyx mori.* Biochemical and Biophysical Research Communications 246(2): 388-392.

Kim S R, Hong M Y, Park S W, Choi K H, Yun E Y, Goo T W, Kang S W, Suh H J, Kim I, Hwang J S. 2010. Characterization and cDNA cloning of a cecropin-like antimicrobial peptide, papiliocin, from the swallowtail butterfly, *Papilio xuthus*. Molecules and Cells 29: 419-423.

Kockum K, Faye I, Hofsten P V, Lee J Y, Xanthopoulos K G, Boman H G. 1984. Insect immunity, Isolation and sequence of two cDNA clones corresponding to acidic and basic attacins from *Hyalophora cecropia*. The EMBO Journal 3(9): 2071-2075.

Kreil, G. 1994. In Antimicrobial Peptides (Ciba Foundation Symposium 186), pp.77-90, John Wiley & Sons, Inc., Chichester.

Lambert J, Keppi E, Dimarcq J L, Wicker C, Reichhart J M, Dunbar B, Lepages P, Van Dorsselaer A, Hoffmann J, Forthergill J, Hoffmann D. 1989. Insect immunity: isolation from immune blood of the dipteran *Phormia terranovae* of two insect antibacterial peptides with sequence homology to rabbit lung macrophage bactericidal peptides. Proceedings of National Academy of Sciences 86(1): 262-266.

Lamberty M, Ades S, Uttenweiler-Joseph S, Brookhart G, Bushey D, Hoffmann J A, Bulet P. 1999. Insect immunity: Isolation from the lepidopteran Heliothisvirescens of a novel insect defensin with potent antifungal activity. Journal of Biological Chemistry 274(14): 9320-9326.

Lamberty M, Caille A, Landon C, Tassin-Moindrot S, Hetru C, Bulet P, Vovelle F. 2001. Solution structures of the antifungal heliomicin and a selected variant with both antibacterial and antifungal activities. Biochemistry 40(40): 11995-12003.

Lavine M D, Strand M R. 2002. Insect hemocytes and their role in immunity. Insect biochemistry and molecular biology 32(10): 1295-309.

Lekha G, Vijayagwori E, Sirigineedi S, Sivaprasad V, Ponnuvel K M. 2014. Differential level of host gene expression associated with nucleopolyhedrovirus infection in silkworm races of *Bombyx mori*. International Journal of Industrial Entomology 29: 145-152.

Lekha G, Gupta T, Awasthi A K, Murthy G N, Trivedy K, Ponnuvel K M. 2015. Genome wide microarray-based expression profiles associated with BmNPV resistance and susceptibility in Indian silkworm races of *Bombyx mori*. Genomics 106: 393-403.

Lemaitre B, Hoffmann J. 2007. The host defense of *Drosophila melanogaster*. Annual review of Immunology 25: 697-743.

Li Z, Ma Y, Liu X, Li Y, Dai F. 2019. Antimicrobial peptides in silkworm. *Animal Biology 69*(4): 391-410.

Liu X, Guo C, Huang Y, Zhang X, Chen Y. 2015. Inhibition of porcine reproductive and respiratory syndrome virus by Cecropin D in vitro. Infection, Genetics and Evolution 34: 7-16.

Lundstrom A, Liu G, Kang D, Berzins K, Steiner H. 2002. *Trichoplusia ni* gloverin, an inducible immune gene encoding an antibacterial insect protein. Insect Biochemistry and Molecular Biology 32(7): 795-801.

Lu D, Geng T, Hou C, Huang Y, Qin G, Guo X. 2016. *Bombyx mori* cecropin A has a high antifungal activity to entomopathogenic fungus *Beauveria bassiana.* Gene 583(1): 29-35.

Mackintosh J A, Gooley A A, Karuso P H, Beattie A J, Jardine D R, Veal D A. 1998a. A gloverin-like antibacterial protein is synthesized in *Helicoverpa armigera* following bacterial challenge. Developmental and Comparative Immunology 22(4): 387-399.

Mahlapuu M, Björn C, Ekblom J. 2020. Antimicrobial peptides as therapeutic agents: Opportunities and challenges. Critical reviews in biotechnology 40(7): 978-92.

Manniello M D, Moretta A, Salvia R, Scieuzo C, Lucchetti D, Vogel H, Sgambato A, Falabella P. 2021. Insect antimicrobial peptides: Potential weapons to counteract the antibiotic resistance. Cellular and Molecular Life Science 78: 4259-4282.

Makwana P, Dubey H, Pradeep A N R, Sivaprasad V, Ponnuvel K M, Mishra R K. 2021. Dipteran endo parasitoid infestation actively suppressed host defense components in hemocytes of silkworm *Bombyx mori* for successful parasitism. Animal Gene 22: 200118.

Makwana P, Kamisi R, Katsuhiko I, Bindu S. 2023. Diversity of antimicrobial peptides in silkworm. Life 13(5): 1161.

Mastore M, Quadroni S, Caramella S, Brivio M F. 2021. The Silkworm as a Source of Natural Antimicrobial Preparations: Efficacy on Various Bacterial Strains. Antibiotics 10(11): 1339.

Matsuyama K, Natori S. 1998. Purification of three antibacterial proteins from the culture medium of NIH-Sape-4, an embryonic cell line of *Sarcophega peregrina.* Journal of Biological Chemistry 263(32): 17112-17116.

Meng X, Zhu F, Chen K. 2017. Silkworm: A Promising Model Organism in Life Science. Journal of Insect Science 17(5): 97.

Minakhina S, Steward R. 2010. Hematopoietic stem cells in *Drosophila*. Development 137(1):27-31.

Moore A J, Beazley W D, Bibby M C, Devine D A. 1996. Antimicrobial activity of cecropins. Journal of Antimicrobial and Chemotherapy 37(6): 1077-1089.

Montano A M, Tsujino F, Takahata N, Satta Y. 2011. Evolutionary of peptidoglycan recognition proteins in vertebrate innate immune system. BMC Evolutionary Biology 11(1): 1-10.

Moretta A, Scieuzo C, Petrone A M, Salvia R, Manniello M D, Franco A, Lucchetti D, Vassallo A, Vogel H, Sgambato A, Falabella P. 2021. Antimicrobial peptides: A new hope in biomedical and pharmaceutical fields. Frontiers in Cellular and Infection Microbiology p 453.

Mrinal N, Nagaraju J. 2008. Intron loss is associated with gain of function in the evolution of the gloverin family of antibacterial genes in *Bombyx mori.* Journal of Biological Chemistry 283(34): 23376-23387.

Nakajima Y, Qu X M, Natori S. 1987. Interaction between liposomes and sarcotoxin IA, a potent antibacterial protein of *Sarcophaga peregrina* (flesh fly). Journal of Biological Chemistry 262(4): 1665-1669.

Nayak T, Mandal S M, Neog K, Ghosh A K. 2017. Characterization of a gloverin-like antimicrobial peptide isolated from muga silkworm, *Antheraea assamensis.* International journal of Peptides Research and Therapeutics 24: 337-346.

Nesa J, Sadat A, Buccini D F, Kati A, Manda A K, Franco O L. 2020. Antimicrobial peptides from *Bombyx mori:* a splendid immune defense response in silkworms. Royal Society of Chemistry Advances 10(1): 512-523.

Noorwala M, Mohammad F V, Ahmad V U, Sener B. 1994. A bidesmosidic triterpene glycoside from the roots of *Symphytum officinale*. Phytochemistry 36(2): 439-443.

Oizumi Y, Hemmi H, Minami M, Asaoka A, Yamakawa M. 2005. Isolation, gene expression and solution structure of a novel moricin analogue, antibacterial peptide from a lepidopteran insect, *Spodoptera litura.* Biochimica et Biophysica Acta (BBA)-proteins and Proteomics 1752(1): 83-92.

Ouyang, L.; Xu, X.; Freed, S.; Gao, Y.; Yu, J.; Wang, S.; Ju, W.; Zhang, Y.; Jin, F. Cecropins from Plutella xylostella and Their Interaction with Metarhizium anisopliae. PLoS ONE 10: e0142451.

Okada M, Natori S. 1985. Primary structure of sarcotoxin I, an antibacterial protein induced in the hemolymph of *Sarcophaga peregrina* (flesh fly) larvae. Journal of Biological Chemistry 260(12): 7174-7177.

Park S I, An H S, Chang B S, Yoe S M. 2013. Expression, cDNA cloning, and characterization of the antibacterial peptide cecropin D from *Agrius convolvuli*. Animal Cells and System 17(1): 23-30.

Pillai A, Ueno S, Zhang H, Lee J M, Kato Y. 2005. Cecropin P1 and novel nematode cecropins: a bacteria-inducible antimicrobial peptide family in the nematode *Ascaris suum*. Biochemical Journal 390(1): 207-214.

Ponnuvel K M, Yamakawa M. 2002. Immune responses against bacterial infection in *Bombyx mori* and regulation of host gene expression. Current Science 83: 447-454.

Pradeep A N R, Anita J, Awasthi A K, Babu M A, Geetha M N, Arun H K, Chandrashekhar S, Rao G C. 2013. Activation of autophagic programmed cell death and innate immune gene expression reveals immuno-competence of integumental epithelium in *Bombyx mori* infected by a dipteran parasitoid. Cell and Tissue Research 352: 371-385.

Ramzah N H, Yenn T W, Lee W H, Loo C Y, Tan W N, Ring L C. 2023. Antimicrobial peptides, an alternative antimicrobial agent against multi-drug-resistant microbes: Source, application, and potential. In Advancements in Materials Science and Technology Led by Women 9: 235-259.

Ravindar G, Ragamalika G and Nagaraja Rao P. 2015. Analysis of proteins profile and antibacterial activity in haemolymph of Eri silkworm, *Samia cynthia ricini* after bacterial inoculation. International Journal of Advanced Research 3(1): 186-192.

Ravi C, Jeyashree A, Devi K R. 2011. Antimicrobial Peptides from insects: an overview. Research in Biotechnology 2(5).

Ratcliffe N A. 1985. Invertebrate immunity- a primer for the non-specialist. Immunology Letters 10(5): 253-270.

Ribeiro C, Brehélin M. 2006. Insect haemocytes: what type of cell is that?. Journal of insect physiology 52(5): 417-29.

Rizki T M, Rizki R M. 1984. The cellular defense system of *Drosophila melanogaster*. Insect ultrastructure 2: 579-604.

Russell V W, Dunn P E. 1996. Antibacterial proteins in the midgut of *Manduca sexta* during metamorphosis. Journal Insect Physiology 42(1): 65-71.

Saviane A, Romoli O, Bozzato A, Freddi G, Cappelletti C, Rosini E, Cappellozza S, Tettamanti G, Sandrelli F. 2018. Intrinsic

antimicrobial properties of silk spun by genetically modified silkworm strains. Transgenic Research 27: 87-101.

Schmid-Hempel, P. 2005. Evolutionary ecology of insect's immune defenses. Annual Review of Entomology 50: 529-551.

Silvestro L, Axelsen P H. 2000. Membrane-induced folding of cecropin A. Biophysical Journal 79(3): 1465-1477.

Song L, Wang F, Dong S, Hu C, Hua X, Xia Q. 2015. Paralytic peptide activates insect humoral immune response via epidermal growth factor receptor. Peptide, 71: 20-27.

Strand M R. 2008. The insect cellular immune response. Insect science 15(1): 1-4.

Srisailam S, Arunkumar A I, Wang W, Yu C, Chen H M. 2000. Conformational study of a custom antibacterial peptide cecropin B1: implications of the lytic activity. Biochimica et Biophysica Acta (BBA)-Protein Structure and Molecular Enzymology 1479(1-2): 275-285.

Srisailam S, Kumar T K, Arunkumar A I, Leung K W, Yu C, Chen H M. 2001. Crumpled structure of the custom hydrophobic lytic peptide cecropin B3. European Journal of Biochemistry 268(15): 4278-4284.

Steiner H, Hultmark D, Engstrom A, Bennich H, Boman H G. 1981. Sequence and specificity of two antibacterial proteins involved in insect immunity. Nature 292(5820): 246-248.

Suttmann H, Retz M, Paulsen F, Harder J, Zwergel U, Kamradt J, Wullich B, Unteregger G, Stockie M, Lehmann J. 2008. Antimicrobial peptides of the Cecropin-family show potent antitumor activity against bladder cancer cells. BMC Urology 8(1): 1-7.

Tan J, Xu M, Zhang K, Wang X, Chen S, Li T, Xiang Z, Cui H. 2013. Characterization of hemocytes proliferation in larval silkworm, Bombyx mori. Journal of insect physiology 59(6): 595-603.

Tanaka H, Ishibashi J, Fujita K, Nakajima Y, Sagisaka A, Tomimoto K, Suzuki N, Yoshiyama M, Kaneko Y, Iwasaki T, Sunagwa T, Yamaji K, Asaoka A, Mita K, Yamakawa M. 2008. A genome-wide analysis of genes and gene families involved in innate immunity of *Bombyx mori*. Insect Biochemistry and Molecular Biology 38(12): 1087-1110.

Uddin S J, Shilpi J A, Nahar L, Sarker S D, Göransson U. 2021. Natural antimicrobial peptides: Hope for new antibiotic lead molecules. Frontiers in Pharmacology 12: 640938.

Vanzolini T, Bruschi M, Rinaldi A C, Magnani M, Fraternale A. 2022. Multitalented synthetic antimicrobial peptides and their antibacterial, antifungal and antiviral mechanisms. Int. J. Mol. Sci. 23: 545.

Volkoff A N, Rocher J, d'Alencon E, Bouton M, Landais I, Quesada-Moraga E, Vey A, Fournier P, Mita K, Devauchelle G. 2003. Characterization and transcriptional profiles of three Spodoptera frugiperda genes encoding cysteine-rich peptides: A new class of defensin-like genes from lepidopteran insects? Gene 319: 43-53.

Wang M, Zhou Z, Li S, Zhu W, Hu X. 2021. Identification and characterization of antimicrobial peptides from butterflies: an integrated bioinformatics and experimental study. Frontiers in microbiology 12: 720381.

Wang Y P, Lai R. 2010. Insect antimicrobial peptides: structures, properties and gene regulation. Zoological research 31(1): 27-34. DOI : 10.3724/SP.J.1141.2010.01027.

Wang D, Qiu X, Wang H. Qiao K, wang K. 2010. Reduced fitness associated with spinosad resistance in *Helicoverpa armigera*. Phytoparasitica 38: 103–110.

Wan J, Zhou X, Zhou X. 2012. A review of innate immunity of silkworm, *Bombyx mori*. African Journal of Agricultural Research 8(20): 2319-2325.

Wen H, Lan X, Cheng T, He N, Shiomi K, Kajiura Z, Zhou Z, Xia Q, Xiang Z, Nakagaki M. 2009. Sequence structure and expression pattern of a novel anionic defensin-like gene from silkworm (*Bombyx mori*). Molecular Biology reports 36: 711-716.

Wu Qinghua, Patoˇcka Jiˇrí, Kuˇca Kamil Kuˇca. 2018. Insect antimicrobial peptides, a mini review. Toxins 10(11): 461.

Xiao Y, Hughes A L, Ando J, Mastuda Y, Cheng J F, Skinner-Noble D, Zhang G. 2004. A genome-wide screen identifies a single β-defensin gene cluster in the chicken: implications for the origin and evolution of mammalian defensins. BMC Genomics 5: 1-11.

Xu X X, Zhong X, Yi H Y, Yu X Q. 2012. *Manduca sexta* gloverin binds microbial components and is active against bacteria and fungi. Developmental and Comparative Immunology 38(2): 275-284.

Yamano Y, Matsumoto M, Sasahara K, Sakamoto E, Morishima I. 1998. Structure of genes for cecropin A and an inducible nuclear protein that binds to the promoter region of the genes from the silkworm, *Bombyx mori.* Bioscience, biotechnology and biochemistry 62(2): 237-241.

Yamakawa M, Tanaka H. 1999. Immune proteins and their gene expression in the silkworm, *Bombyx mori.* Developmental & Comparative Immunology 23(4-5): 281-289.

Yang W, Cheng T, Ye M, Deng X, Yi H, Huang Y, Tan X, Han D, Wang B, Xiang Z, Cao Y, Xia Q. 2011. Functional divergence among silkworm antimicrobial peptide paralogs by the activities of recombinant proteins and the induced expression profiles. PLOS ONE 6(3): e18109.

Yi H Y, Chowdhury M, Huang Y D, Yu X Q. 2014. Insect antimicrobial peptides and their applications. Applied microbiology and biotechnology 98: 5807-5822.

Yoe S M, Kang C S, Han S S, Bang I S. 2006. Characterization and cDNA cloning of hinnavin II, a cecropin family antibacterial peptide from the cabbage butterfly, *Artogeia rapae*. Comparative Biochemistry and Physiology- Biochemistry and Molecular Biology 144(2): 199-205.

Yun J, Lee D G. 2016. Cecropin A-induced apoptosis is regulated by ion balance and glutathione antioxidant system in *Candida albicans*. IUBMB Life 68(8): 652-662.

Zhao W Y, Dong B R, Zhou Y. 2005. In vitro antimicrobial activity of defensins from rabbit neutrophils against *Pseudomonas aeruginosa* and its multiple-drug-resistance strains. Sichuan da xue xue bao. Yi xue ban 36(1): 83-85.

Zhu Y, Johnson T J, Myers A A, Kanost M R. 2003. Identification by subtractive suppression hybridization of bacteria-induced genes expressed in *Manduca sexta* fat body. Insect biochemistry and molecular biology 33(5): 541-559.

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