Journal of Advances in Biology & Biotechnology
9(3): 1-15, 2016; Article no.JABB.27539
ISSN: 2394-1081

SCIENCEDOMAIN *international*
www.sciencedomain.org

A Review on Antimicrobial Peptides from *Bombyx mori* L and Their Application in Plant and Animal Disease Control

Sebrin Jahan Islam[1,2], Sukanya Bezbaruah[1,2] and Jatin Kalita[1*]

[1]Biological Science and Technology Division, CSIR-North East Institute of Science and Technology, Jorhat- 785006, Assam, India.
[2]Academy of Scientific and Innovative Research, Chennai-600113, Tamil Nadu, India.

Authors' contributions

This work was carried out in collaboration between all authors. Author SJI designed the study, wrote the protocol and wrote the first draft of the manuscript. Authors JK and SJI managed the analyses of the study and revision of the manuscript. Authors SB and SJI managed the literature searches. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/JABB/2016/27539
Editor(s):
(1) Ali Movahedi, Forest Genetics and Biotechnology in the college of Forest Resources and Environment, Nanjing Forestry University, Nanjing, Jiangsu, China.
Reviewers:
(1) Anonymous, University of Ferrara, Ferrara, Italy.
(2) Manuel Soriano-García, Universidad Nacional Autónoma de México, Mexico.
(3) John George Hardy, Lancaster University, UK.
Complete Peer review History: http://www.sciencedomain.org/review-history/16090

Review Article

Received 7th June 2016
Accepted 2nd September 2016
Published 8th September 2016

ABSTRACT

Antimicrobial peptides (AMPs) are low molecular weight biologically active molecules having exciting properties. A wide variety of AMPs have been isolated from various sources like plants, animals, mammals, insects and microorganisms. The most familiar structure is represented by α-helical conformation in organic solutions or disulfide stabilized β-sheet with or without α-helical domains present. Despite of striking diversity in structure and chemical nature, all of them possess antimicrobial activity. This fundamental property makes them as a promising candidate compared to chemical antibiotics. In the near future attempts should be made to generate AMPs with higher antimicrobial activity and broad range of microbe action. In this review, focus is given on the AMPs found in the silkworm *Bombyx mori,* with some findings on the innate immunity responses (both cellular and humoral). This review also summarizes six classes of antimicrobial peptides (cecropin, defensin, attacin, lebocin, moricin and gloverin) found in *B. mori* and their mode of actions against diverse range of pathogenic microbes.

**Corresponding author: E-mail: kalitajk74@gmail.com;*

Keywords: Antimicrobial peptides; Bombyx mori L; disease control.

ACRONYMS

Acronym : Full meaning
AMP : Antimicrobial peptide
TrEMBL : Translations of European Molecular Biology Laboratory
AMSDd : Antimicrobial Sequence Database
APD : Antimicrobial Peptide Database
ANTIMIC : A Database of Antimicrobial Sequemces
ESBL : Extended Spectrum beta lactamase
WHO : World Health Organization
HIV : Human Immunodeficiency Virus
AIDS : Acquired Immuno Deficiency Syndrome
RelB : RelishB
BmDp : Bombyx mori Defensin like protein
EST : Expressed Sequence Tags
RTPCR : Real Time Polymerase Chain Reaction
GSTmDP : Glutathione- S- Transferase Maltose Binding Protein
NMR : Nuclear Magnetic Resonance
LPS : Lipopolysaccharide
BmGlv : BombyxmoriGloverin
NF-kB : Nuclear factor KappaB
RNAi : Ribonucleic acid Interference
OmpC : Outer membrane protein C
OmpA : Outer Membrane Protein A
OmpF : Outer membrane protein F
OmpR : Outer membrane protein R
DNA : Deoxyribonucleic acid
Ets : Erythroblast Transformation Specific
GUS : B-Glucuronidase

1. INTRODUCTION

Bombyx mori L silkworms (commonly known as Mulberry Silkworm) are one of the major domesticated silkworms for producing commercial silk. The silkworm feeds on the Mulberry plant, which have different species under family Moraceae. Besides the production of good quality silk of commercial interest, the silkworm has been exploited for its different bioactive properties. Recently, there have been many studies carried out on possible antimicrobial peptides from *Bombyx mori* L, wherein the major focus is on peptides of antimicrobial origin that exhibit different mechanism of action and lesser side effects. Many of enormous antimicrobial peptides (AMPs) have been produced from insects, mammals, reptiles and plants to protect against microbial infections and environmental changes [1].

2. WHAT ARE ANTIMICROBIAL PEPTIDES?

Antimicrobial peptides are small molecular weight proteins having broad range of activity against bacteria, fungi and viruses. These biologically active peptides are synthesized by vast number of organisms as an essential factor for innate immune response. These peptides are considered as probable candidate for forthcoming drugs, because of their broad range of activity, lesser toxicity and decreased resistance development by target cells. The smaller size of AMPs helps in the rapid diffusion and secretion outside the cells, which is mandatory for evoking immediate response against pathogenic microbes. Other necessary factors such as size, charge, hydrophobicity, amphiphatic stereo geometry and self-association with the biological membrane are critically essential for their broad spectrum antimicrobial activities [2]. Out of the knowledge gained from the past suggests that discovery of antimicrobial peptides makes natural antibiotics a key element for the generation of novel drugs for the treatment of bacterial and fungal infections [3-6] Moreover, the wide spectrum of antimicrobial activity of these peptides makes them potentially suitable in the treatment of cancer [7] and viral [8-10] or parasitic infections [11]. Lipid composition variation between

prokaryotic and eukaryotic membranes is the main targets for AMPs. The antibacterial activity of some peptides lies in their ability to interfere with a specific mechanism of the microbial cell without affecting similar mechanisms present in the cells of the infected organism. A huge number of AMPs reported in the literature are already listed in the publicly available databases including Swissprot and TrEMBL, AMSDd, APD and ANTIMIC. The Antimicrobial Peptide Database (APD) offers a network to conclude the antimicrobial activity of any submitted sequence, based on a simple residue analysis and count method and some favourable statistical information on peptide sequence, function and structure. AMPs are commonly designated as peptides having less than 100 amino acid residues with an overall positive charge (usually +2 to +9). They have presence of multiple lysine and arginine residues and a substantial portion (≥30% or more) of hydrophobic residues [12]. They are mostly cationic in nature but few of them are anionic. Cationic peptides share the common property to fold into amphiphatic membranes, which is usually induced upon interaction with membrane or membrane mimics. Besides having antimicrobial properties against Gram-positive and Gram-negative bacteria, they are also effective towards fungi [13] and protozoa [14] with micromolar or submicromolar minimal inhibitory concentrations (MIC) [15].

3. WHY ANTIMICROBIAL PEPTIDES?

The rate of spread of multi resistant microbial strains is becoming a source of lethal infections. The antimicrobial drug resistance is due to the high rate of genetic mutations of bacteria, making them resistant to treatments formerly effective. Antimicrobial drug resistance arises due to high rate of genetic mutation in microbes that make them resistant to formerly used antimicrobial agents which were found to be effective earlier. The property of bacterial strains for rapid transfer of their genes suggests that bacterial resistance to antibiotics occurs quickly in the evolution of bacterial development [16]. According to antimicrobial resistance report by World Health Organization (2014), common type of drug resistant bacteria includes *Escherichia coli*, *Klebsiella pneumonia*, *Staphylococcus aureus*, *Neisseria gonorrhoeae*, *Streptococcus pneumonia*, *Shigella* species that are acquired resistant against common antibiotics like cephalosporins, fluoroquinolones, carbapenems, methicillin, penicillin, including resistance conferred to extended spectrum beta lactamases (ESBL). The World Health Organization (WHO) report also stated that this serious threat is no longer a prediction for the future; it is happening right now in every region of the world and has the potential to affect anyone, of any age, in any country. The rise of fungal infections at the same time in the decades is due to increase of immune-compromised patients, including HIV/AIDS patients, oncology patients with chemotherapy induced neutropenia and transplant recipients who are receiving immunosuppressive therapy [17]. To combat with the present multi drug resistance predicament, the use of AMPs from insects becoming a major area of interest for the discovery of new antibiotics which is biologically active, less toxic and reduction of resistance. Insect AMPs become a major area of interest for the discovery of new antibiotics which are biologically active and less toxic to combat with the present multi drug resistance predicament.

4. ANTIMICROBIAL PEPTIDES FROM *B. mori*

4.1 Cecropins

Cecropins are cationic antimicrobial peptides, first isolated from the immunized hemolymph of the giant silk moth, *Hyalophora cecropia* [18,19]. In insects three principles cecropins are present, *viz.* A, B and D having a length of 35 to 37 residues which lacks cysteine with a strong basic N-terminal linked to a neutral C-terminal by a flexible glycine proline link [20]. Cecropin was also recognized as a part of immune response in two silkworm *Bombyx mori* and *Antherea peryni*. Many families of cecropin have been isolated in lepidopteran and dipteran insects. Members of cecropins family include, sarcotoxin-I [21], papiliocin [22], stomoxyn [23], hinnavin [24], SB-37 and Shiva (synthetic derivatives of cecropins) [25]. Cecropins have broad range of antimicrobial activity against Gram-positive and Gram-negative bacteria and also to fungi. The antimicrobial property of cecropin is governed by the amidation at the C-terminus and amidation is necessary for the interaction of cecropin with liposomes [26]. Besides antimicrobial property cecropin and cecropin derivatives (SB-37 and Shiva) are active against parasites like *Plasmodium* and *Trypanosom* [27-29] and can inhibit the replication of HIV-1 virus [30] and proliferation of cancerous cells [31]. The antibacterial peptide gene of cecropin XJ was cloned in Pyes2/CT/a expression vector and

expressed in *Saccharomyces cerevisiae* INCScI strain. This recombinant protein had shown strong antimicrobial activities against Gram-positive and Gram-negative bacteria. This study also suggests that yeast can be used system for the large production of this protein by genetic engineering methods [32]. A promoter from *Bombyx mori* cecropin A1 (Cec A1) was cloned and characterized to study the transcriptional control of the antimicrobial peptide during immune challenges. From the deletion and mutation constructs it was affirmed that regulatory region is kB motif which increases the activity of the promoter [33]. Cecropins maintain a random coil structure in aqueous solution but transformed to an alpha-helical structure in hydrophobic environments. *Hyalopohora cecropia* cecropin A having 37 residues contains two helical regions in residues 5-21 and 24-37 [34] and sarcotoxin IA (39 residues) display an N-terminal amphiphatic alpha-helix (residues 3-23) and a more hydrophobic C-terminal alpha helix (residues 28-38) joined by a hinge region (residues 24-27) [35].

4.2 Defensins

Defensins are cationic antimicrobial peptides (4 kDa) together with six conserved cysteine residues that form three intra-molecular disulfide bridges. These peptide are mainly subdivided based on their spacing pattern of the cysteines into five groups, particularly invertebrate, plant, α, β and θ subfamilies [36]. Insect defensin was first detected in flesh fly, *Sarcophoga peregrine* which contain six cysteine residues as *sapecins* [37] and in the immunized larvae of *Phormia terranovae* as cationic peptides [38]. It includes phormicins, sapecins [39,40] royalisin [41] and spodoptericin [42]. In contrast to antibacterial defensin only few antifungal defensins have been reported, drosomycin from *Drosophila*; heliomycin from the tobacco budworm *Heliothis virecens* [43], gallerimycin from greater wax moth larvae *Galleria mellonella* [44], termicin from the isopteran *P. spiniger* [45] and Alo13 from the harlequin beetle *Acrocinus longimanus* (Coleoptera) [46]. A gene related to defensin called BmDefensinA was discovered in the silkworm genome. Reports suggest that 5'-upstream regulatory region of gene encoding BmDefensinA contains cis elements such as NF-kβ binding site, an IL-6 responsive element and the GATA motif. BmDefA open reading frame encodes a propeptide which consist a 22 residue signal peptide, a 34- residue propeptide and a 36-residue mature peptide having a molecular weight of 4 kDa. The presence of six cysteine motif in the mature peptide suggests a characteristics feature of insect defensin and isoelectric point was found out to be 4.12 which suggest that it is a novel anionic defensin. This is highly transcribed in the hemocyte, silk gland, head, and ovary of the silkworm larvae and in the fat body of early stage pupae and moth. BmDefA is strongly induced by immune challenge which represents its important role in both immunity and metamorphosis [47]. BmDefensinB a homolog of defensin was indentified in the silkworm *Bombyx mori.* The presence of six cysteine residues in conserved sequence is a crucial characteristic of all defensins. BmDefensinB has low amino acid similarity (about 27%) with that of BmDefensinA. The BmDefensinB gene was expressed in the fat body and it was found that it is strongly activated by bacteria such as *Escherichia coli* and *Bacillus subtilis*, and also by *Bauveria bassiana*. On the other hand, the BmDefensinA gene was expressed at lesser extent. The expression of BmDefensinB gene was strongly stimulated by the Rel protein RelB and Relish. The results of the study suggested that BmDefensinB gene expression is controled through both the Toll and Imd pathway. This strongly suggests that BmDefensinB plays a crucial role in regulating immune reaction against both bacteria and fungi in *B. mori.* This study also demonstrates that BmDefensinB is the first antimicrobial peptide gene activated by *B. bassiana* [48]. *Bombyx mori* defensin like peptide (BmDp) was identified by wdS20658 cDNA from the *Bombyx mori* Expressed sequence Tags (EST) database constructed from non-immune challenged cells in *B. mori*. This peptide was deposited in Genbank in 2005, having Genbank accession number DQ118523. It was found that wdS20658 cDNA has consists of six cysteines [C…CXXXC…C…CXC] and showed high identity of 75% with spodoptericin. Semiquantitave RT-PCR analysis showed that gene expression of BmDp was inducible by bacterial injection immunization and it was highest after 8h of immunization. This finding suggested that BmDp is associated with immune response against bacteria. A purified form of the recombinant GSTmDp fusion protein when assayed did not show any significant activity against bacteria and fungi [49]. When mode of action of defensin was tested with the help of recombinant insect defensin using *Micrococcus luteus* as test organism it was reported that defensin disrupts the permeability barrier of cytoplasmic membrane of *M. luteus* which results in the loss

of cytoplasmic potassium, partial depolarization of the inner membrane, decrease in cytoplasmic ATP and inhibits respiration. It was observed that permeability changes in the membrane reflect the formation of channels in the cytoplasmic membrane by defensin oligomers. These results were strongly supported by patch-clamp experiments that show that insect defensins form channels in giant liposome [50].

4.3 Moricin

Moricin was isolated from the hemolymph of *Bombyx mori* which was found out to be active against *Staphylococcus aureus* [51]. The basic nature of moricin may be important for the attachment of positively charged peptides to the negatively charged bacterial surfaces through electrostatic interaction [52]. Moreover, the presence of amphiphatic α-helix is mainly responsible for antibacterial activity. There is a presence of charged amino acids at intervals of three of four amino acid residues in the N-terminal half of moricin, implying a distinctive structure in antibacterial proteins containing the amphiphatic which shows a characteristic α-helix [53]. Although, the main target for moricin is bacteria but it shows slight antifungal activity against some strains of yeast. Despite the fact that both moricins and cecropins act against several species of bacteria, moricin tends to have higher activity against Gram-positive bacteria. Bacterial membranesare mainly targeted by moricin and the C-terminal fragment is mainly responsible for attaching with the membrane by which it changes the permeability of the membrane by N –terminal amphiphatic a-helix. Further, the absence of modifications in the moricin such as α-amidation of C termini in the hydroxylation of Lys residues in *B. mori* cecropins, the O-glycosylation of Thr residues in proline-rich antibacterial peptides or formation of intermolecular disulfide bonds in defensins gives a exclusive property to moricin for the production of the peptide by chemical synthesis or by biotechnology using suitable protein expression vectors [54]. cDNA encoding moricins have been identified in *M. sexta* [54] *Spodoptera litura* [55], *G. mallonella* [56], *H. armigera* [57], *S. exigua*, *H. virescens* and *Hyblaea peura* and *Bombyx mori*. From the results of molecular cloning, cDNA encoding moricin differ in the coding and non-coding regions which results to one amino acid substitution in the putative signal peptide.

Gene expression of both moricin 1 and moricin 2 was found to be induced upon bacterial infection, which strongly suggests that gene dosage is an efficient strategy in insects to protect themselves from bacterial invasion. Two dimensional 1H nuclear magnetic resonance (NMR) spectroscopy reveals the presence of a unique structure comprising of a α-helix containing eight turns along the entire length of the peptide except for four N-terminal residues and six C-terminal. Electrostatic surface map confirmed that amphiphatic N-terminal of the α-helix is important for the increase in permeability of membrane to kill the bacteria [58].

4.4 Gloverins

Gloverin is a basic insect inducible antibacterial protein isolated from the pupae of giant silk moth *Hyalaphora cecropia*. It contains large number of glycine residues (18.5%) but no cysteine residues and has a distinct amino acid sequence that reveals no strong degree of identity with any known proteins [59]. Till now gloverin have been identified only in Lepidoptera including, *H. armigera* [60], *T. ni* [61], *G. mellonella* [62], *Antheraea mylitta* [63], *M. sexta* [64], *Diatraea saccharialis* [65], *S. exigua* [66], and *B. mori* [67]. Gloverins were found to be active against *E. coli,* mutant strains of (Df21f2, D21 and D22) containing Lipopolysaccharide (LPS). Gloverin from *T. ni* is active against virus and *S. exigua* is active against *Flavobacterium* sp., On the other hand it is inactive against *E. coli* strains with smooth LPS. Results of EST and whole genome shotgun analysis showed the presence of four gloverin like genes, BmGlv 1-4 homologous to *Hyalaphora gloveri* gloverin. Northern Blot and RT-PCR analysis revealed that BmGlov genes have been induced in the fat body when challenged with *E. coli,* but the induction was less with yeast *Candida albicans.* In silico sequence analysis reveals that presence of a motif which is homologous to the Nuclear Factor κB (NF-kB) binding site in the upstream of each BmGlov gene. A recombinant form of BmGlov genes were also expressed in the Baculovirus virus system and found that all the BmGlov1-4 genes significantly inhibit the growth of *E. coli.* Bmgloverin 1 is considered as the ancestor among the four Bmgloverin genes, gloverin genes 2-4 are derived from the duplication. Knockdown expression studies of *B. mori* gloverin-2 performed using RNAi, showed reduction in the hatching rate of the embryos [68]. Bmgloverin 1 was found to be expressed only in larva but not in adult gonads; on the other hand gloverins 2-4 was expressed in adult but not in larval gonads [69].

4.5 Attacin

Attacins were first isolated from the hemolymph of bacteria immunized *H. cecropia* pupae having a molecular mass of 20-23 kDa. Attacins were divided into two groups basic (A-D) and acidic (E-F) having isoelectric points (pI 5.7 - 8.3). The different isoforms of attacin were generated by the post translational modifications of two parental pro-attacins sequences. It was reported that attacins have a very high content of aspartic acid, glycine, alanine and unusual levels of phenylalanine and threonine. Basic attacins reported to have a high content of threonine, glutamic acid, lysine and tryptophan whereas acidic attacins have more aspartic acid, isoleucine and arginine. Attacins were synthesized as pre-pro-proteins containing a signal peptide, a pro-peptide (P-domain), an N-terminal attacin domain, followed by two glycine-rich domains (G1 and G2 domains) [70]. A conserved motif of amino acids RXXR is present at the N-terminal pro-peptide of attacins [71] which is recognized by furin-like enzymes [72]. This finding indicates that mature attacins are produced by processing of pro-attacins by furin-like enzymes. Antibacterial assays of attacin It reveals that apart from *Escherichia coli* Acinetobacter *calcoacetius* and *Pseudomonas maltophilia* isolated from the gut of a Chinese oak silkworm were found to be sensitive to attacins. It was suggested that attacins act only on growing cells and cause chain formation, unlike cecropins which causes destruction of bacterial membranes. When cDNA of *Bombyx mori* was hybridized with *Hyalophoa cecropia* attacin it was observed that cDNA consist of 846 nucleotides and it encodes attacin precursor protein. The mature peptide had 70.4, 68.3 and 18.8% indentity in amino acid sequence with that of *Hyalaphora cecropia* acidic and basic attacins and *Sarcophoga peregrine* sarcotoxin IIA. The presence of two subdomains in the G domains in *Bombyx mori* and *H. cecropia* attacins and *S. peregrine* sarcotoxin IIA, suggests that common amino acid residues in the subdomains are conserved during evolution and seems to play an important role in the activity of antibacterial proteins. The expression level of attacin quickly induced when injected with *Escherichia coli* cells into *B. mori* larvae and continued for 48 hours in the fat body and hemocytes [73]. The mechanism present in attacin which bring about the alteration in the structure and permeability of the outer membrane of *Escherichia coli* is related with specific inhibition of the synthesis of several outer membrane proteins including OmpC, OmpA, OmpF and LamB. The effect of inhibition is manifested by the reduction in the steady-state mRNA levels and as a minimum in part the result of block in transcription of the analogous genes. The transcription of the OmpC and OmpF genes is under the genetic control of the regulatory locus OmpB, composed by OmpR, encoding a cytoplasmic DNA-binding protein, and envZ, encoding a cytoplasmic membrane localized environmental sensor protein. In a mutant strain called HSK24, which have a deletion in ompR and envZ and has a mutated chromosomal OmpC on the other hand carries an intact ompC clone on the plasmid pHSK21 suggest that reduction in the amounts of OmpC and OmpA comparable to that was observed in wild-type strains after treatment with attacin. It was concluded that attacin effect on Omp genes requires neither OmpR nor envZ. These data suggest the presence of an unknown system in *E. coli* for the transcriptional regulation of a large set of outer membrane proteins not known to be co-ordinately regulated instead attacin helps in the regulation of the system [74].

4.6 Lebocin

It is an antibacterial peptide rich in proline and O-glycosylated consisting of 32 amino acids isolated from by the hemolymph of silkworm, *B. mori* immunized with *Escherichia coli.* The glycosylation in the amino acid residue (15-Thr) is an essential feature for antibacterial activity. The primary structure and antibacterial activity of this peptide mainly coincides with abaecin [75]. Two different analogues of lebocin, lebocin 1 and lebocin 2 have indentical amino acid residues but they differ on length of their sugar chains on the threonine residues. It was reported that glycosylated threonines of these antibacterial proteins contain different sugars that is *N-acetylgalactosamine* and galactose in Lebocin 1 and *N-acetylgalactosamine* in Lebocin 2.-. Amino acid sequence of lebocin3 showed one amino acid replacememt at 16 Leu and 15 Thr. Lebocin 4 amino acid sequence shows similarity with other members of lebocin. When lebocin was incubated with liposome preparation, it causes the leakage of entrapped glucose under low ionic conditions, which suggests that bacterial membrane is a target for lebocin. Biological significance of lebocin is still remains doubtful because it shows very weak antibacterial activity under physiological conditions and requires low ionic strength for full expression. Further, Lebocin 3 has combine

effect with cecropin D which suggest that antimicrobial protein works co-operatively in the immunity of *B. mori* [76].

cDNA encoding lebocin precursors have been identified in Lepidopteran species like *M. sexta* [77], *Trichoplusia ni* [78], *Pseudoplusia includens* [79], *Pieris rapea* (Genbank accession number: JN587806), *H. viresecns* (genbank accession number: FJ546346), and *Antheraea peryni* (Genbank accession number: EU557311, EU57312 and DQ666499). All these lebocin precursors along with *Bombyx mori* precursors are proline rich peptides having 4 to 6 prolines residues present in N-termini of mature precursor protein. Precursors of *Bombyx mori* consists of extra 32-residue peptides with 7 prolines which are closed to the C-termini [80]. From the cotransfection experiment with the silkworm cell line, the overexpression of an erythroblast transformation-specific (Ets) family of protein called BmEts is essential for the elevation of activity of lebocin promoters. However, BmEts has no effect on cecropins 1, cecropins D, attacin and moricin promoters. BmEts activity towards lebocin promoter was found to be depends on at least two kB elements and the proximal GGAA/T motif located in the 5-upstream. In addition, it also collectively enhances the *E. coli* or BmRelish1-d2 stimulated lebocin promoter activation [81].

5. APPLICATIONS OF INSECT AMPS IN PLANT DISEASE CONTROL

Several diseases caused by bacteria, fungi and viruses affect the yield of crop production, which results in losses and decreasing the quality of agricultural crops. In most cases the application of biological and chemical treatments, when feasible is not successful to control the diseases. One approach to improve the plant defence against the pathogens has been possible by use of genetic engineering. Antimicrobial proteins, peptides and lysozymes naturally found in insects are now a potential source of plant resistance. It was reported that among insect AMPs, attacins, defensins and cecropins have high potential of defence to microbial infections and protect plants. Transgenic expression of genes of antimicrobial peptides, especially insect AMPs in plants, may confer a successful strategy to combat phytopathogens in modern plant protection.

Cecropins have been expressed in transgenic tobacco confers resistance to *Pseudomonas syringae*pv *tabaci* [82]. Cecropin B expressed in tobacco, enhanced the resistance against bacterial wilt caused by *Pseudomonas solanacearum* [83] and in rice it leads to increase in resistance to *Xanthomanas oryzae* [84].

The expression of *Bombyx mori* cecropin B in transgenic rice, when fused with a signal peptide sequence of chitinase showed strong resistance to *X. oryzae* pv *oryzae*. It has been reported that growth of plant pathogenic fungi like *Pseudomonas syringae, Xanthomonas campestris* or *Erwiniw carotova* was completely inhibited by concentration of cecropin B between 5 and 15 µg/mL (1.3-3.9 µM). Cecropins inhibit the plant pathogenic fungi like *Penicillium digitatum* or *Phytophthora infestans* when applied at a concentration of 15.6 µM [85].

Expression of cecropin in transgenic rice and tomato conferred resistance against bacterial and fungal pathogen [86]. A derivative of cecropin called SB-37 showed resistance against *E. carotovora* [87] and *P. syringae* pv. *tabaci* [88]. A hybrid peptide of cecropin-melltin when tested against several plant pathogenic bacteria *P. syringae, Erwinia amylovora, Xanthomonas vesicatoria)*, oomycetes and fungi like *(P. infestans, M. oryzae*) exhibit a broad range of antimicrobial activity and inhibit the growth of pathogens. Transgenic tobacco plants expressing the cecropin-mellitin antimicrobial hybrid peptide of 29 amino acid acids found to be resistant against *F. solani* and did not show toxic effects on plants. A construct called CEMA of cecropin-mellitin hybrid conferred resistance to *F. solani, Phytophthora cactorum* and *E. carotovora* when expressed in transgenic tobacco plants. Another class of antimicrobial peptides called sarcotoxin IA was found to be involved in the disturbance of protein gradient and membrane potential of bacteria [89]. However, sarcotoxin IA, when expressed in the transgenic tobacco plants under the regulation of constitutive promoterCaMV35S, the amount of peptide formed was less. So, in order to increase the expression level of the peptide it was fused with β-glucuronidase (GUS) and the transgenic protein produced in tobacco was found to be increased. When the leaf extracts from the transgenic tobacco plants checked for antimicrobial activity it actively inhibit the growth of *E. coli* suspension cultures. However, transgenic plants showed abnormal plants [90].

Gallerimycin an antifungal peptide expressed in transgenic tobacco using *Agrobacterium*

tumefaciens as vector, conferred resistance against *Erysiphe cichoracearum* and *Sclerotinia minor* [91]. Transgenic barley plants expressing metchnikowin showed enhance resistance against to powdery mildew as well as *Fusarium* head blight and root rot [92]. Attacin expression in transgenic potato enhanced its resistance to bacterial infection by *E. carotovora* subsp. Atrospetica [93]. Attacin was also expressed in transgenic pear and apple (Royal Gala), expression of genes has considerably enhanced resistance to *E. amylovora in vitro* and growth chamber test [94]. A reduction in disease called fire blight has been observed in transgenic apples expressing attacin in field tests.

A study on transgenic apples reports that attacins when targeted to intracellular space, where *E. amylovora* multiplies before infection, had notably reduced fire blight, also in apple plants with low attacin production levels. Transgenic citrus expressing the attacin E (attE) had reduced the susceptibility of 'Duncan' grapefruit to the *Elsinoe fawcetti*, citrus scab fungal agent [95].

6. APPLICATIONS OF INSECT AMPS IN ANIMAL DISEASE CONTROL

Defensin isolated from the beetle *Allomyrina dichotoma* showed sensitivity towards pathogenic strains of *Staphylococcus aureus.* These pathogenic strains were resistant to antibiotic such as methicillin [96]. An analogue of cecropin called D2A21, when applied to the infected wounds of rats showed fruitful results, with 100% survival of rats with infected wounds compared to the 50% survival rate with control [97]. Halocidin, a peptide isolated from the tunicate was found to be active against resistant strains of *S. aureus* and *Pseudomonas aeruginosa* [98]. Antimicrobial peptides were reported which were to use in the treatment of ophthalmic and dental problems. With the increase in use of contact lenses the problems associated with the cornea also gets boost up. Analogues of cecropin called Shiva 11, d5c and Hecate were all examined for their potentiality abilities to disinfect contact lenses as well as contact lens solutions. It was found out that d5c have the ability to increase capacity of existing contact lens sterilizing solutions to sterilise contact lenses [99]. Shiva 11 and Hecate were both able to kill bacterial isolates from infected contact lenses. Alloferons a peptide isolated from the blow fly *Calliphora vicina* have the ability to prevent mortality in mice that have been infected by influenza virus by either interfering with the viral assembly or viral attachment to the cell. It was found out that naturally occurring alloferon, as well as synthetic alloferon was able to slow the tumor growth. It was unable to eliminate cancer cells at high concentrations. A derivative of mellitin called Hecate, produced by altering the charge distribution of mellitin, while at the same time retaining its three dimensional structure exhibits antiviral activity against herpes simplex virus-1. When applied at low concentrations it reduces the plaque formation. It was able to prevent (HSV-1) induced cell fusion and virus spread, with no cytotoxic effects [100].

Hecate was found to be toxic towards breast cancer cells. Hecate's efficiency has been increased by creating Hecate hormone conjugates. By conjugating Hecate to hormones, whose receptors are found on the surface of cancer cells e.g. luteinizing hormone, the cell selectivity of hecate can be increased. Both cecropin and mellitin were able to inhibit the production of HIV-1 in infected cells. These peptides acquire this by decreasing both HIV-1 transcription and the number of viral gene products [101]. When mellitin was produced by changing a few L-amino acids with D-enantiomers, these peptides were no longer haemolytic but were very effective against tumor cells. Some of the antimicrobial peptides also reported to have anti-inflammatory effects. Synthetic peptides, Arg-Leu-Tyr-Leu-Arg-Ile-Gly-Arg-Arg-NH_2 (peptide A) and Arg-Leu-Arg-Leu-Arg-Ile-Gly-Arg-Arg-NH_2 (peptide B), derived from the beetle *Allomyrina dichotoma* defensin were found to protect mice from endotoxic shock. It exerts its effect by inhibiting the production of tumor necrosis factor α. The process involves the peptide prevensiLPS from binding to LPS receptors on the surface of macrophage Tachyplesin III from horse shoe crab was able to kill a multidrug resistant strain called *P. aeruginosa.* In conjugation with conventional antibiotics the effect of Tachyplesin III was extremely enhanced tachyplesin was also able to protect the mice from endotoxic shock following bacterial lysis [102]. Thantin hemipteran peptide was found to be potent against multidrug resistant isolates of *Enterobacter aerogenes* and *Klebsiella pneumoniae*. Thanatin also restored the antibiotic susceptibility of these resistant isolates. This must be achieved by making the membrane of the bacteria more porous to antibiotics or interfering with the bacteria's ability to expel specific character of the cell membrane [103].

Table 1. Application of antimicrobial peptides in plant disease control

Antimicrobial peptide	Active against	Expression in transgenic plant
Cecropin	*Pseudomonas syringae* pv. *tabaci*	Tobacco
Cecropin B	*Pseudomonas solanacearum*	Tobacco
	Xanthomanas oryzae	Rice
	X. oryzae pv. *oryzae*	
	Penicillium digitatum	Tomato
	Phytophthora infestans	
Cecropin A	*Fusarium* species	Rice
	P. infestans	
	Magnaporthe oryzae	
	Botrytis cinerea	
Sarcotoxin IA	*Rhizoctonia solani*	Tobacco
	Pythium aphanideratum	
	Phytophthora nicotianae var. *nicotianae*	
	Orobanche aegyptica	Tomato
Gallerimycin	*Golovinomyce cichoracearum*	Tobacco
	Sclerotinia minor	
	Erysiphe cichoracaerum	
Metchnikowin	*Fusarium graminearum*	Barley
Attacin	*E. carotovora* subsp. Atrospetica	Potato
	E. amylovor	Pear
	E. amylovor	Apple
	Elsinoë fawcettii	Grapefruit

Table 2. Primary sequence of antimicrobial peptides present in silkworm *Bombyx mori*

Name of the peptide	Primary structure of the peptide
Cecropin A	RWKLFKKIEKVGRNVRDGLIKAGPAIAVIGQAKSL
Cecropin B	RWKIFKKEKMGRNIRDGIVKAGPAIEVLGSAKAI
Cecropin D	GNFFKDLEKMGQRVRDAVISAAPAVDTLAKAKALGQ
Defensin	ATATATTTAGTTTGAGCCGTGTAACGAGTGAACATGAAGGGGGTTTATTAATTTT CACCCTAGTTCTAGTATACGTTGCTTCGACCTGGGCTTCACTAGATGCAGCTGA TGAAGTTCGAGTTATGAACGTGGAATCCCAAAGGCTGTTTCGATCCAGGAGGG CCTTACCATGTGCGAAGAAGAGCTGTGACAGCTGGTGCCGGAGATTGGATATT CCAGGCGGAGAATGTGTAACAAAGTGGAAATGCTCCTGTAATTGGATGCAGAT TGACAAATAATAATATTTCTCTATCTCTCATCAGAACAATACTGTTGGTTATTACT TAAAATGTTTATCTTTTTAAAAAAAAAAAAAAAAA
Moricin (1KV4)	AKIPIKAIKTVGKAVGKGLRAINIASTANDVFNFLKPKKRKH
Lebocin 1	DLRFLYPRGKLPVPTPPPFNPKPIYIDMGNRY
Lebocin 2	DLRFLYPRGKLPVPTPPPFNPKPIYIDMGNRY
Lebocin 3	DLRFLYPRGKLPVPTPPPFNPKPIYIDMGNRY
Lebocin 4	DLRFLYPRGKLPVPTPPPFNPKPIYIDMGNRY
Attacin	QAGSFTVNSDGTSGAALKVPLTGNDKNVLSAIGSADFNDR HKLSAASAGLALDNVNGHGLSLTGTTRIPGFGEQLGVAGKV NLFHNNNHDLSAKAFAIRNSPSAIPNAPNFNTLGGGVDYM FKQKVGASLSAAHSDVINRNDYSAGGKLNLFRSPSSSLDF NAGFKKFDTPFYRSSWENNVGFSFSKFF

7. CONCLUSION

The severe problems related with multi drug resistant microorganisms have created crucial demand for the development of alternative therapeutics. Concurrently with the increase in resistance to commercially available antibiotics, there is a serious need for novel, effective

therapeutics with lesser or no side effects. However, AMPs are the promising candidate for the production of new generation antibiotics. At the industrial levels, many companies are concentrating on the development of AMPs at both preclinical and clinical stages. These AMPs are produced by virtually all species as a part of their immediate non-specific defence. The worth of these peptides in clinical sectors consists of their broad spectrum activity; low propensity for resistance development, ease of synthesis but the questions arises with their high cost, limited stability. Several procedures are have been implemented to enhance AMPs with favourable activity, ranging from the addition of non natural amino acids and high-throughput screening for multimerization of linear sequences. Even though it is a known fact that resistance may evolve at any time bacterial populations are regularly exposed to elevated levels of AMPs, this concern should not discourage their development in near future.

ACKNOWLEDGEMENTS

The authors are thankful to the Director, CSIR-NEIST, Jorhat for his support and also acknowledge the receipt of financial support from Department of Biotechnology, Govt. of India.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Soares JW, Mello CM. Antimicrobial peptides: A review of how peptide structure impacts antimicrobial activity. Proc. SPIE 5271, Monitoring Food Safety, Agriculture and Plant Health. 2004;20.
   DOI: 10.1117/12.516171
2. Pushpanathan M, Gunasekaran P, Rajendhran J. Antimicrobial peptides: Versatile biological properties. Int J Pept. 2013.
   DOI: 10.1155/2013/675391
3. De Lucca, JA. Antifungal peptides: Potential candidates for the treatment of fungal infections. Expert Opin Inv Drug. 2000;9(2):273-299.
4. Hancock RE. Cationic antimicrobial peptides: Towards clinical applications. Expert OpinInv Drug. 2000;9(8):1723-1729.
5. Welling MM, Paulusma-Annema A. Balter HS, Pauwels EK, Nibbering PH. Tenehtium-99m labeled antimicrobial peptide discriminate between bacterial infection and sterile inflammations. Eur J Nucl Med. 2000;27(3):292-301.
6. Claude PS. Antifungal proteins. Appl Environ Microbiol. 2001;67:2883-2894.
7. Tanaka K, Fujimoto Y, Suzuki M, Suzuki Y, Ohtake T, Saito H, Kohgo Y. P-I3 - kinase p85 is a target molecule of proline-rich antimicrobial peptide to suppress proliferation of ras-transformed cells. Jpn J Cancer Res. 2001;92(9):959-967.
8. Chinchar VG, Wang J, Murti G, Carey C, Rollins-Smith L. Inactivation of frog virus 3 and channel catfish virus by esculentin-2P and ranatuerin-2P, two antimicrobial peptides isolated from frog skin. Virology. 2001;288(2):351-357.
9. Andersen JH, Osbakk SA, Vorland LH, Traavik T, Gutteberg TJ. Lactoferrin and cyclic lactoferricin inhibit the entry of human cytomegalovirus into human fibroblasts. Antiviral Res. 2001;51(2):141-149.
10. Chernysh S, Kim SI, Beckeer G, Pleskach VA, Filatova NA, Anikin VB, Platonov VG. Bulet P. Antiviral and antitumor peptides from insects. Proceedings of the National Academy of Science USA. 2002; 99(20):12628-12632.
11. Vizioli J, Salzet M. Antimicrobial peptides: New weapons to control parasitic infections? Trends in Parasitology. 2003; 19.
12. Andrea G, Giovanna P, Silvia FN. Antimicrobial peptides: An overview of a promising class of therapeutics. Cent Eur Journal Biol. 2007;2(1):1-33.
13. Noorwala M, Mohammad FV, Ahmad VU, Sener B. Bidesmosidic triterpenoidal saponins from the roots of *Symphytum officinale*. Phytochemistry. 1994;36(2):439-43.
14. Aley SB, Zimmerman M, Hetsko M, Selsted ME, Gillin FD. Killing of *Giardia lamblia* by cryptdins and cationic neutrophil peptides. Infect Immun. 1994;62(12): 5397–403.
15. Hancock RE, Lehrer R. Cationic peptides: A new source of antibiotics. Trends Biotechnol. 1998;16(2):82–88.
16. Bulet P, Stocklin R. Insect antimicrobial peptides: Structures, properties and gene regulation. Protein Pept Lett. 2005;12(1): 3-11.

17. Venkatesan P, Perfect JR, Myers SA. Evaluation and management of fungal infections in immunocompromised patients. Dermatol Ther. 2005;18(1):44-57.
18. Hultmark D, Engstrom A, Bennich H, Kapur R, BomanHG. Insect immunity: Isolation and structure of cecropin D and four minor antibacterial components from *Cecropia pupae*. European Journal of Biochemistry. 1982;127(1):207–217.
19. Steiner H, Hultmark D, Engstrom A, Bennich H, Boman HG. Sequence and specificity of two antibacterial proteins involved in insect immunity. Nature. 1981; 292(5820):246–248.
20. Moore AJ, Beazley WD, Bibby MC, Devine DA. Antimicrobial activity of cecropins. J Antimicrob Chemother. 1996;37(6):1077-1089.
21. Okada M, Natori S. Primary structure of sarcotoxin I, an antibacterial protein induced in the hemolymph of *Sarcophaga peregrina* (flesh fly) larvae. J Biol Chem. 1985;260(12):7174–7177.
22. Kim SR, Hong MY, Park SW, Choi KH, Yun EY, Goo TW, Kang SW, Suh HJ, Kim I, Hwang JS. Characterization and cDNA cloning of a cecropin-like antimicrobial peptide, papiliocin, from the swallowtail butterfly. *Papilio xuthus*. Mol Cells. 2010; 29(4):419–423.
23. Boulanger N, Munks RJ, HamiltonJV, Vovelle F, Brun R, Lehane MJ, Bulet P. Epithelial innate immunity. A novel antimicrobial peptide with antiparasitic activity in the blood-sucking insect *Stomoxys calcitrans*. J Biol Chem. 2002; 277(51):49921–49926.
24. Yoe SM, Kang CS, Han SS, Bang IS. Characterization and cDNA cloning of hinnavin II, a cecropin family antibacterial peptide from the cabbage butterfly, *Artogeia rapae*. Comp Biochem Physiol B Miol Biol. 2006;144(2):199–205.
25. Jaynes JM, Burton CA, Barr SB, Jeffers GW, Julian GR, White KL, Enright FM, Klei TR, Laine RA. *In vitro* cytocidal effect of novel lytic peptides on *Plasmodium falciparum* and *Trypanosoma cruzi*. FASEB J. 1988;2(13):2878–2883.
26. Nakajima Y, Qu XM, Natori S. Interaction between liposomes and sarcotoxin IA, a potent antibacterial protein of *Sarcophaga peregrina* (flesh fly). J Biol Chem. 1987; 262(4):1665–1669.
27. Arrowood MJ, Jaynes JM, Healey MC. In vitro activities of lytic peptides against the sporozoites of *Cryptosporidium parvum*. Antimicrob Agents Chemother. 1991; 35(2):224–227.
28. Barr SC, Rose D, Jaynes JM. Activity of lytic peptides against intracellular *Trypanosoma cruzi* amastigotes *in vitro* and parasitemias in mice. J Parasitol. 1995;81(6):974–978.
29. Boisbouvier J, Prochnicka-Chalufour A, Nieto AR, Torres JA, Nanard N, Rodriguez MH, Possani LD, Delepierre M. Structural information on a cecropin-like synthetic peptide, Shiva-3 toxic to the sporogonic development of *Plasmodium berghei*. Eur J Biochem. 1998;257(1):263–273.
30. Chen HM, Wang W, Smith D, Chan SC. Effects of the anti-bacterial peptide cecropin B and its analogs, cecropins B-1 and B-2, on liposomes, bacteria, and cancer cells. Biochimica Biophysica Acta. 1997;1336(2):171–179.
31. Suttmann H, Retz M, Paulsen F, Harder J, Zwergel U, Kamradt J, Wullich B, Unteregger G, Stockle M, Lehmann J. Antimicrobial peptides of the Cecropin-family show potent antitumor activity against bladder cancer cells. BMC Urology. 2008;8:5.
32. Xia L, Zhang F, Liu Z, Ma J, Yang J. Expression and characterization of cecropinXJ, a bioactive antimicrobial peptide from *Bombyx mori* (Bombycidae, Lepidoptera) in *Escherichia coli.* Exp and Ther Med. 2013;5(6):1745–1751.
33. Hua XT, Ma XJ, Xue RJ, Cheng TC, Wangand F, Xia QY. Characterization of the *Bombyx mori Cecropin A1* promoter regulated by IMD pathway. J Insect Sci. 2015;12.
DOI: 10.1111/1744-7917.12210
34. Holak TA, Engstrom A, Kraulis PJ, Lindeberg G, Bennich H, Jones TA, Gronenborn AM, Clore GM. The solution conformation of the antibacterial peptide cecropin A: A nuclear magnetic resonance and dynamical simulated annealing study. Biochemistry. 1988; 27:7620–7629.
35. Iwai H, Nakajima Y, Natori S, Arata Y, Shimada I. Solution conformation of an antibacterial peptide, sarcotoxin IA, as determined by 1H-NMR. Eur J Biochem. 1993;217(2):639–644.
36. Xiao Y, Hughes AL, Ando J, Mastuda Y, Cheng JF, Skinner-Noble D, Zhang G. A genomewide screen indentifies a single β-defensin gene cluster in the chicken:

implications for the origin and evolution of mammalian defensins. BMC Genomics. 2004;5:56.

37. Matsuyama K, Natori S. Purification of three antibacterial proteins from the culture medium of NIH Sape-4, an embryonic cell line of *Sacrophoga peregrine*. J Biol Chem. 1988;263(32):17112-17116.
38. Lambert J, Keppi E, Dimarcq JL, Wicker C, Reichhart JM, Dunbar B, Lepage P, Van DA, Hoffmann J, Fothergill J, Hoffmann D. 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. Proc Natl Acad Sci U.S.A. 1989;86:262–266.
39. Matsuyama K, Natori S. Purification of three antibacterial proteins from the culture medium of NIH Sape-4, an embryonic cell line of *Sacrophoga peregrine*. J Biol Chem. 1988;263(32):17112-17116.
40. Yamada K, Natori S. Purification, sequence and antibacterial activity of two novel sapecin homologues from Sarcophaga embryonic cells: Similarity of sapecin B to charybdotoxin. Biochem J. 1993;291(Pt 1):275–279.
41. Fujiwara S, Imai J, Fujiwara M, Yaeshima T, Kawashima T, Kobayashi K. A potent antibacterial protein in royal jelly. Purification and determination of the primary structure of royalisin. J Biol Chem. 1990;265(19):11333–11337.
42. Volkoff AN, Rocher J, d'Alencon E, Bouton M, Landais I, Quesada-Moraga E, Vey A, Fournier P, Mita K, Devauchelle G. Characterization and transcriptional profiles of three *Spodoptera frugiperda* genes encoding cysteine-rich peptides. A new class of defensin-like genes from lepidopteran insects? Gene. 2003;319:43–53.
43. Lamberty M, Ades S, Uttenweiler-Joseph S, Brookhart G, Bushey D, Hoffmann JA, Bulet P. Insect immunity. Isolation from the lepidopteran *Heliothis virescens* of a novel insect defensin with potent antifungal activity. J Biol Chem. 1999;274(14):9320–9326.
44. Lee YS, Yun EK, Jang WS, Kim I, Lee JH, Park SY, Ryu KS, Seo SJ, Kim CH, Lee IH. Purification cDNA cloning and expression of an insect defensin from the great wax moth, *Galleria mellonella*. Insect Mol Biol. 2004;13(1):65–72.
45. Lamberty M, Zachary D, Lanot R, Bordereau C, Robert A, Hoffmann JA, Bulet P. Insect immunity constitutive expression of a cysteine-rich antifungal and a linear antibacterial peptide in a termite insect. J Biol Chem. 2001b; 276(6):4085–4092.
46. Barbault F, Landon C, Guenneugues M, Meyer JP, Schott V, Dimarcq JL, Vovelle F. Solution structure of Alo-3: A new knottin-type antifungal peptide from the insect *Acrocinus longimanus.* Biochemistry. 2003;42(49):14434-14442.
47. Wen H, Lan X, Cheng T, He N, Shiomi K, Kajiura Z, Zhou Z, Xia Q, Xiang Z, Nakagaki M. Sequence structure and expression pattern of a novel anionic defensin-like gene from silkworm (*Bombyx mori*). Mol Bio Rep; 2008.
DOI: 10, 1007/s11033-008-9233-4
48. Yoichi K, Hiromitsu T, Jun I, Takashi I, Minoru Y. Gene expression of a novel defensin antimicrobial peptide in the silkworm, *Bombyx mori*. Biosci Biotechnol Biochem. 2008;72(9):2353-2361.
49. Song KJ, Bo-Ryung Park, So Y. Kim, Kwang S. Park. Molecular characterization of anionic defensin-like peptide in immune response of silkworm, *Bombyx mori* L. (Lepidoptera). Genes & Genomics. 2010;32:447-453.
50. Stephane C, Alexandre G, Charles H, Jules AH, Lucienne L. Insect defensin, an inducible antibacterial peptide, forms voltage-dependent channels in *Micrococcus luteus*. J Biol Chem. 1993; 26:19239-19245.
51. Hara S, Yamakawa M. Moricin, a novel type of antibacterial peptide isolated from the silkworm, *Bombyx mori*. J Biol Chem. 1995;270(50):29923-29927.
52. Christensen B, Fink J, Merrifield R, Mauzerall D. Channel-forming properties of cecropins and related model compounds incorporated into planar lipid membranes. Proc Natl Acad Sci USA. 1988; 85(14):5072–5076.
53. Cociancich S, Ghazi A, Hetru C, Hoffmann JA, Letellier L. Insect defensin, an inducible antibacterial peptide, forms voltage-dependent channels in *Micrococcus luteus*. J Biol Chem. 1993; 268(26):19239-19245.
54. Seiichi Hara, Minoru Yamakawa. Moricin, a novel type of antibacterial peptide isolated from the silkworm, *Bombyx mori*. J Biol Chem. 1995;270(50):29923–29927.

55. Oizumi Y, Hemmi H, Minami M, Asaoka A, Yamakawa M. Isolation, gene expression and solution structure of a novel moricin analogue, antibacterial peptide from a lepidopteran insect, *Spodoptera litura*. Biochim Biophys Acta. 2005;1752(1):83–92.
56. Brown SE, Howard A, Kasprzak AB, Gordon KH, East PD. The discovery and analysis of a diverged family of novel antifungal moricin-like peptides in the wax moth *Galleria mellonella*. Insect Biochem Mol Biol. 2008;38(2):201–212.
57. Wang Q, Liu Y, He HJ, Zhao XF, Wang JX. Immune responses of *Helicoverpa armigera* to different kinds of pathogens. BMC Immunol. 2010;11-9.
58. Axen A, Carlsson A, Engstrom A, Bennich H. Gloverin, an antibacterial protein from the immune hemolymph of *Hyalophora pupae*. Eur J Biochem. 1997;247(2):614–619.
59. Axen A, Carlsson A, Engstrom A, Bennich H. Gloverin, an antibacterial protein from the immune hemolymph of *Hyalophora pupae*. Eur J Biochem. 1997;247(2):614–619.
60. Mackintosh JA, Gooley AA, Karuso PH, Beattie AJ, Jardine DR, Veal DA. A gloverin-like antibacterial protein is synthesized in *Helicoverpa armigera* following bacterial challenge. Dev Comp Immunol. 1998;22(4):387–399.
61. Lundstrom A, Liu G, Kang D, Berzins K, Steiner H. *Trichoplusia ni* gloverin, an inducible immune gene encoding an antibacterial insect protein. Insect Biochem Mol Biol. 2002;32(7):795–801.
62. Seitz V, Clermont A, Wedde M, Hummel M, Vilcinskas A, Schlatterer K, Podsiadlowski L. Identifcation of immunorelevant genes from greater wax moth (*Galleria mellonella*) by a subtractive hybridization approach. Dev Comp Immunol. 2003;27(3):207–215.
63. Gandhe AS, Arunkumar KP, John SH, Nagaraju J. Analysis of bacteria-challenged wild silk moth, *Antheraea mylitta* (Lepidoptera) transcriptome reveals potential immune genes. BMC Genomics. 2006;7:184.
64. Abdel-latief M, Hilker M. Innate immunity: Eggs of *Manduca sexta* are able to respond to parasitism by *Trichogramma evanescens*. Insect Biochem Mol Biol. 2008;38(2):136–145.
65. Silva JL, Barbosa JF, Bravo JP, Souza EM, Huergo LF, Pedrosa FO, Esteves E, Daffre S, Fernandez MA. Induction of a gloverin-like antimicrobial polypeptide in the sugarcane borer *Diatraea saccharalis* challenged by septic injury. Braz J Med Biol Res. 2010;43(5):431–436.
66. Hwang J, Kim Y. RNA interference of an antimicrobial peptide, gloverin, of the beet armyworm, *Spodoptera exigua*, enhances susceptibility to *Bacillus thuringiensis*. J Invertebr Pathol. 2011;108:194–200.
67. Kawaoka S, Katsuma S, Daimon T, Isono R, Omuro N, Mita K, Shimada T. Functional analysis of four Gloverin-like genes in the silkworm, *Bombyx mori*. Arch Insect Biochem Physiol. 2008;67:87–96.
68. Yi HY, Deng XJ, Yanga WY, Zhouc CZ, Caoa Y, Yub XQ. Gloverins of the silkworm *Bombyx mori*: Structural and binding properties. Insect Biochem Mol Biol. 2013; 43(7):612–625.
69. Mrinal N, Nagaraju J. Intron loss is associated with gain of function in the evolution of the gloverin family of antibacterial genes in *Bombyx mori*. J Biol Chem. 2008;283:23376–23387.
70. Hedengren M, Borge K, Hultmark D. Expression and evolution of the drosophila attacin/diptericin gene family. Biochem Biophys Res Commun. 2000;279(2):574–581.
71. Gunne H, Hellers M, Steiner H. Structure of preproattacin and its processing in insect cells infected with a recombinant baculovirus. Eur J Biochem. 1990; 187(3):699–703.
72. Devi L. Consensus sequence for processing of peptide precursors at monobasic sites. FEBS Lett. 1991; 280:189–194.
73. Sugiyama M, Kuniyoshi H, Kotani E, Taniai K, Kadono-Okuda K, Kato Y, Yamamoto M, Shimabukuro M, Chowdhury S, Xu J, Choi SK, Katoaka H, Suzuki A, Yamakawa M. Characterization of a *Bombyx mori* cDNA encoding a novel member of the attacin family of insect antibacterial proteins. Insect Biochem Mol Biol. 1995; 25(3):385-92.
74. Carlsson A, Engstrom P, Palva ET, Bennich H. Attacin, an antibacterial protein from *Hyalophora cecropia*, inhibits synthesis of outer membrane proteins in *Escherichia coli* by interfering with omp gene transcription. Infect Immun. 1991; 59(9):3040-3045.

75. Casteels P, Ampe C, Riviere L, Van DJ, 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(2):381–386.
76. Ponnuvel KM, Yamakawa M. Immune responses against bacterial infection in *Bombyx mori* and regulation of host gene expression. Special Section: Recent Advances in Silkworm Biology.
77. Rao XJ, Xu XX, Yu XQ. Functional analysis of two lebocin-related proteins from *Manduca sexta*. Insect Biochem Mol Biol. 2012;42(4):231–239.
78. Liu G, Kang D, Steiner H. *Trichoplusia ni* lebocin, an inducible immune gene with a downstream insertion element. Biochem Biophys Res Commun. 2000;269(3):803–807.
79. Lavine MD, Chen G, Strand MR. Immune challenge differentially affects transcript abundance of three antimicrobial peptides in hemocytes from the moth *Pseudoplusia includens*. Insect Biochem Mol Biol. 2005; 35(12):1335–1346.
80. Bao Y, Yamano Y, Morishima I. A novel lebocin-like gene from eri-silkworm, *Samia cynthia ricini*, that does not encode the antibacterial peptide lebocin. Comp Biochem Physiol B Biochem Mol Biol. 2005;140(1):127–131.
81. Tanaka H, Sagikasa A, Fujita K, Furukuwa S, Ishibashi J, Yamakawa M. BmEts upregulates promoter activity of lebocin in *Bombyx mori.* Insect Biochem Mol Biol. 2012;42(7):474-481.
82. Huang Y, Nordeen RO, Di M, Owens LD, McBeath JH. Expression of an engineered cecropin gene cassette in transgenic tobacco plants confers disease resistance to *Pseudomonas syringae* pv. *Tabaci.* Phytopathol. 1997;87(5):494-499.
83. Jaynes J, Nagpala P, Destefano-Beltran L, Huang J, Kim J, Denney T, Cetiner S. Expression of a cecropin B lytic peptide analogue in transgenic tobacco confers enhanced resistance to bacterial wilt caused by *Pseudomonas solanaceum*. Plant Sci. 1993;89:43-53.
84. Sharma A, Sharma R, Imamura M, Yamakawa M, Machii H. Transgenic expression of cecropin B, an antibacterial peptide from *Bombyx mori*, confers enhanced resistance to bacterial leaf blight in rice. FEBS Lett. 2000;484(1):7-11.
85. Alan AR, Earle ED. Sensitivity of bacterial and fungal plant pathogens to the lytic peptides, MSI-99, Magainin II, and Cecropin B. Mol Plant Microbe Interact. 2002;15(7):701–708.
86. Coca M, Penas G, Gomez J, Campo S, Bortolotii C, Messegeur J, Segundo BS. Enhanced resistance to the rice blast fungus *Magnaporthe grisea* conferred by expression of a cecropin A gene in transgenic rice. Planta. 2006;223(3):392–406.
87. Arce P, Moreno M, Gutierrez M, Gebauer M, Dell'Orto P, Torres H, Acuna I, Venegas A, Jordana X, Kalazich J, Hologiue L. Enhanced resistance to bacterial infection by *Erwinia carotovora* subsp. *atroseptica* in transgenic potato plants expressing the attacin or the cecropin SB-37 genes. Amer J of Potato Res. 1999;76:169–177.
88. Jaynes JM, Nagpala P, Destefano-Beltran L, Huang JH, Kim JH, Denny T, Cetiner S. Expression of a cecropin B lytic peptide analog in transgenic tobacco confers enhanced resistance to bacterial wilt caused by *Pseudomonas solanacearum*. Plant Sci. 1993;89:43–53.
89. Yevtushenko DP, Romero R, Forward BS, Hancock RE, Kay WW, Misra S. Pathogen-induced expression of a cecropin A-melittin antimicrobial peptide gene confers antifungal resistance in transgenic tobacco. J Exp Bot. 2005;56(416):1685–1695.
90. Okada M, Natori S. Primary structure of sarcotoxin I, an antibacterial protein induced in the hemolymph of *Sarcophaga peregrina* (flesh fly) larvae. J Biol Chem. 1985;260(12):7174–7177.
91. Langen G, Imani J, Altincicek B, Kieseritzky G, Kogel KH, Vilcinskas A. Transgenic expression of gallerimycin, a novel antifungal insect defensin from the greater wax moth *Galleria mellonella*, confers resistance to pathogenic fungi in tobacco. Biol Chem. 2006;387(5):549-57.
92. Mohammad R, Gregor L, Jafargholi I, Walaa K, Boran A, Diter von W, Karl-Heinz Kogel, Andreas Vilcinskas. Insect peptide metchnikowin confers on barley a selective capacity for resistance to fungal ascomycetes pathogens. J Exp Botany. 2009;60(14):4105–4114.
93. Okamoto M, Mitsuhara I, Ohshima M, Natori S, Ohashi Y. Enhanced expression of an antimicrobial peptide sarcotoxin IA by

GUS fusion in transgenic tobacco plants. Plant Cell Physiol. 1998;39(1):57-63.

94. Arce P, Moreno M, Gutierrez M, Gebauer M, Dell'Orto P, Torres H, Acuna I, Oliger P, Venegas A, Jordana X, Kalazich J, Holuigue L. Enhanced resistance to bacterial infection by *Erwinia carotovora* subsp. *atroseptica* in transgenic potato plants expressing the attacin or the cecropin SB-37 genes. Amer J of Potato Res. 1999;76:169-177.
95. Mondal SN, Dutt M, Grosser JW, Dewdney MM. Transgenic citrus expressing the antimicrobial gene Attacin E (attE) reduces the susceptibility of 'Duncan' grapefruit to the citrus scab caused by *Elsinoe fawcettii*. Eur J Plant Pathol. 2012;133:391–404.
96. 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(10):1005-1011.
97. Chalekson CP, Neumiester MW, Jaynes J. Treatment of infected wounds with the antimicrobial peptide D2A21. J Trauma. 2003;54(4):770-774.
98. Jang WS, Kim HN, Lee YS, Nam MH, Lee IH. Halocidin: A new antimicrobial peptide from hemocytes of the solitary tunicat *Halocynthia aurantium*. FEBS Letters. 2002;521(1-3):81-86.
99. Sousa LB, Mannis MJ, Schwab IR, Cullor J, Houstani H, Smith W, Jaynes J. The use of synthetic cecropin (D5C) in disinfecting contact lens solutions. CLAO J. 1999; 22(2):114-117.
100. Baghain A, Jaynes J, Enright F, Kousoulas K. An amphipathic a-helical synthetic peptide analogue of melittin inhibits herpes simplex virus-1 (HSV-1)-induced cell fusion and virus spread. Peptides. 1997; 18(2):177-183.
101. Wachinger M, Kleinschmidt A, Winder D, Pechmann NV, 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; 79:731-740.
102. Cirioni O, Ghiseli R, Silvestri C, Kamsay W, Orlando F, Mocchegiani F, Matteo FD, Riva A, Lukasiak J, Scalise G, Saba V, Giacometti A. Efficacy of tachyplesin III, colistin, and imipenem against a multiresistant *Psuedomonas aeruginosa* strain. Antimicrobial Agents and Chemotheraphy. 2007;51:2005-2010.
103. Guoqui W, Xiabo F, Linxian L, Hailiang W, Jiaxan D, Wu H, Rui Z, Lixia G, Zilong S, Tao X. Interaction of antimicrobial peptide S-thanatin with lipopolysaccharide in vitro and in an experimental mouse model of septic shock caused by a multidrug-resistant clinical isolate of *Escherichia coli*. 2010;35:250.254.

Peer-review history:
The peer review history for this paper can be accessed here:
http://sciencedomain.org/review-history/16090