Tewfick et al. Egyptian Journal of Biological Pest Control (2018) 28:33
https://doi.org/10.1186/s41938-018-0037-0

Egyptian Journal of Biological Pest Control

RESEARCH Open Access

Detection of antibacterial protein in *Bacillus sphaericus*-treated *Culex pipiens* (Diptera: Culicidae)

Maha K. Tewfick[1*], Waleed M. Serag[2] and Belal A. Soliman[1]

Abstract

Antimicrobial peptides (AMPs) constitute a major arm of defense in mosquitoes against microbes. The purpose of this study was to determine which of the peptides are produced in the mosquito hemolymph after bacterial treatment. *Culex pipiens* (Diptera: Culicidae) larvae, collected from drainage canal in Suez Governorate, Egypt, were treated with LC_{80} of *Bacillus sphaericus* strain 2362 (0.035 ppm). The hemolymph of bacteria-treated *Cx. pipiens* mosquitoes and non-treated mosquitoes as control were extracted and undergo electrophoresis, using a Bio-Rad Mini-protean II cell. In *Cx. pipiens* mosquitoes emerging from bacteria-treated larvae, a band of protein was detected at 165 kDa. This protein was more likely to be Thioester-containing protein 1 (TEP1). Understanding mosquito resistance mechanism to bacterial control is crucial to build up programs to overcome such resistance.

Keywords: Antibacterial protein, *Bacillus sphaericus*, *Culex pipiens*

Background

Mosquito-borne diseases are among the major concerns of public health. Therefore, there is an urgent need to explore every avenue for developing unique control strategies against mosquito-borne diseases (Hill et al., 2005). However, insects are able to protect themselves from attack by pathogens by a rapid and effective arsenal of inducible immune peptides (Lowenberger, 2001). Insects use an arsenal of immune compounds to combat prokaryotic infections, including defensins, cecropins, and proline-rich and glycine-rich peptides (Jayamani et al., 2015, Kaushal et al., 2016). These immune proteins are synthesized in the fat body and in certain types of hemocytes (Lombardo and Christophides, 2016). In mosquitoes, peptides like cecropin, defensin, and gambicin are reported to have inhibitory effect on bacteria, fungi, and parasites (Harikrishna, et al., 2012). This is a major arm of protection in mosquitoes against microbes. Mosquito defensins are rapidly produced in the mosquito hemolymph within 24 h following bacterial inoculation (Lowenberger et al., 1995). Cecropin A is a linear-helical cationic peptide that is produced by both invertebrates and vertebrates (Saugar et al., 2006). Such peptides are reported to obstruct the development and transmission of eukaryotic pathogens (Paily et al., 2007).

Insect AMPs are divided into three groups according to their amino acid sequence and structural features: (i) cecropins which are linear peptides that form α-helix and lack cysteine residues; (ii) defensins which have a characteristic six to eight conserved cysteine residues that form a stabilizing array of three or four intramolecular disulfide bridges and three domains consisting in a flexible amino-terminal loop, a central α-helix, and a carboxyl-terminal antiparallel b-sheet; and (iii) peptides with an overrepresentation of proline and/or glycine residues, e.g., lebocins and moricins (Bulet et al., 1999).

The purpose of this study was to determine which of the peptides are produced in the *Culex pipiens* mosquito hemolymph after *Bacillus sphaericus* bacterial treatment.

Materials and methods

Mosquito rearing

Immature stages of mosquito were collected from a drainage canal in Suez Governorate, Egypt. Fourth instar larvae and emerging adults were identified according to Harbach (2012). Species other than *Cx. pipiens* were discarded. *Culex pipiens* larvae were reared in the laboratory under controlled conditions of temperature

* Correspondence: kamalmaha2000@yahoo.com
[1]Department of Zoology, Faculty of Science, Suez University, Suez, Egypt
Full list of author information is available at the end of the article

(27 ± 2 °C) and relative humidity (70–80%) and a 12L: 12D photoperiod. The third instar larvae of the filial generation were used in the study.

Bacterial strain

Bacillus sphaericus strain 2362 was provided by Abbot Laboratories, North Chicago, IL, USA. A 1% stock suspension was prepared by suspending 1 g of the granular formulation in 100 ml of distilled water. Dilutions were made by adding appropriate volumes of the stock solution to be included in 100 ml of water. All suspensions were prepared fresh for experimental purposes. The susceptibility of the parental generation to *B. sphaericus* preparation was determined by bioassay tests. For bioassay test, 20 third instar larvae were placed in a disposable 250 ml polyethylene cup containing 100 ml of the required concentration of *B. sphaericus*. Each test was performed in triplicate. Mortality was recorded 48 h after exposure. Lethal concentrations for 50 and 80% mortality levels were determined through a log probit regression analysis (Mulla et al., 1988).

Treatment of *Cx. pipiens* larvae with *B. sphaericus*

Two colonies were cultured under identical laboratory conditions. Field-collected larvae were considered the parental generation of each colony. Then, the colony was split into two lines, one subjected to bacterial treatment and the other was cultured without exposure to the bacterium. About 500 third instar larvae in each replicate were treated with the preparation of *B. sphaericus* at a concentration to yield 80% mortality (LC_{80}) for 48 h. About 2000–2500 larvae were treated to obtain enough survivors to yield sufficient progeny for the next generation. After the exposure period, the surviving larvae were removed, rinsed with distilled water, placed in distilled water in enamel pans, and reared to the next generation. The non-treated line was cultured without any exposure to *B. sphaericus*. The hemolymph of adult mosquitoes emerged from treated larvae was used for protein detection. Hemolymph of adults emerged from non-treated larvae was used as control.

Detection of antibacterial protein by electrophoresis

The hemolymph of bacteria-treated *Cx. pipiens* mosquitoes and non-treated ones as control were extracted and undergo electrophoresis. A Bio-Rad Mini-protean II cell was used. The glass plates were assembled according to the manufacturer's instructions. The appropriate volume of the resolving gel was prepared; poured into the gap between the glass plates, leaving sufficient space for pouring the stacking gel; and overlaid with a thin film of isopropanol to form a smooth surface as well as to remove any air bubbles formed on the surface. After the resolving gel has been polymerized completely, in about 30 min, the isopropanol was poured off and the gel surface was washed with distilled water, then the stacking gel was mixed and poured onto the surface of the polymerized resolving gel, and a Teflon comb was inserted immediately without trapping any air bubbles under the comb teeth. More stacking gel solution was added to fill the remaining space. The gel was left about 30 min to achieve complete polymerization. Meanwhile, the samples were mixed with an equal volume of 2× gel loading buffers, and after polymerization of the stacking gel, the Teflon comb was

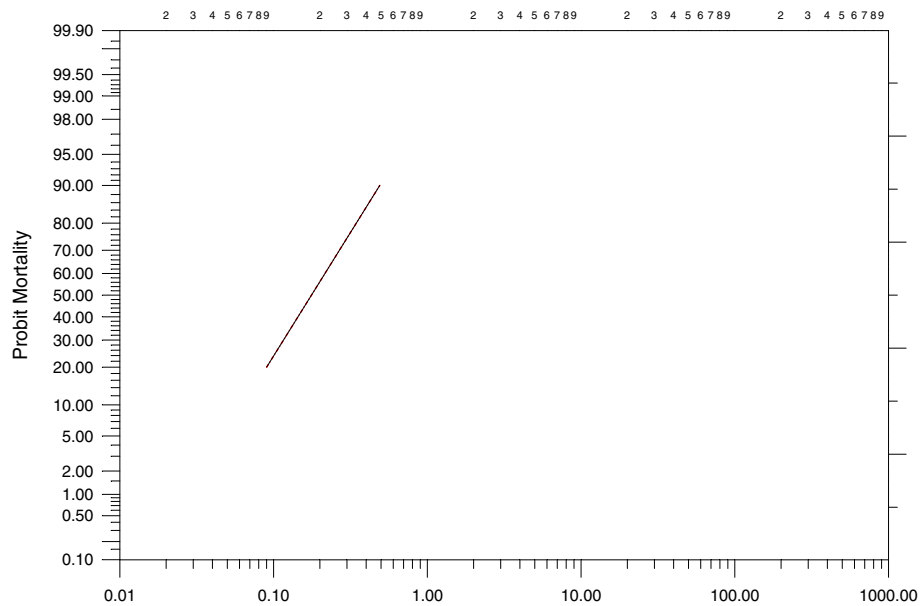

Fig. 1 Log. conc. "ppm." Probit line of *Culex pipiens* to *Bacillus sphaericus* 2362

removed and the wells were washed with distilled water to remove any remnants of polymerized acrylamide. The gel was mounted in the electrophoresis apparatus, and the electrophoresis buffer was added to the top and the bottom reservoirs. The samples as well as the molecular size markers (the BLUelf Prestained Protein Ladder covering a wide range of molecular weights from 3.5 to 245 kDa) were loaded into the wells. The electrophoresis was performed at a constant current of 25 mA for each mini-gel and 50 mA for the regular size gel. The samples were run until the bromophenol blue dye reached the gel front. The gel was then disassembled and stained with coomassie staining solution.

For staining, the gel was immersed in coomassie staining solution and rocked for 30 min, and then, the stain was removed and saved for future use. The gel was destained in destaining solution, until an acceptable background was obtained, and then, it was placed onto a piece of Whatman 3MM filter paper, covered with saran wrap, and dried in a gel dryer under vacuum.

Results and discussion

The sublethal dose of *B. sphaericus* strain 2362 was determined. The calculated median lethal concentration (LC_{50}) was 0.18 ppm (0.15–0.21) ($\acute{\alpha} = 0.05$, $chi^2 = 0.21 < 6$) (Fig. 1). The LC_{80} concentration, calculated to be 0.035 ppm, was subsequently applied on *Cx. pipiens* larvae for further investigation.

In *Cx. pipiens* mosquitoes emerged from bacteria-treated larvae, a band of protein was detected at 165 kDa (Figs. 2 and 3). This band was not found in adult mosquitoes emerged from non-treated larvae and is thus considered to be related to a protein responsible for bacterial resistance. This protein is more likely to be Thioester-containing protein 1 (TEP1), previously reported to be glycosylated and secreted into the body cavity by mosquito immune cells as 165 kDa (Fraiture et al., 2009).

In general, insects utilize germ line-encoded receptors known as pathogen recognition receptors (PRRs) to recognize distinct pathogen-associated molecular patterns (PAMPs) that are either present on the surface of

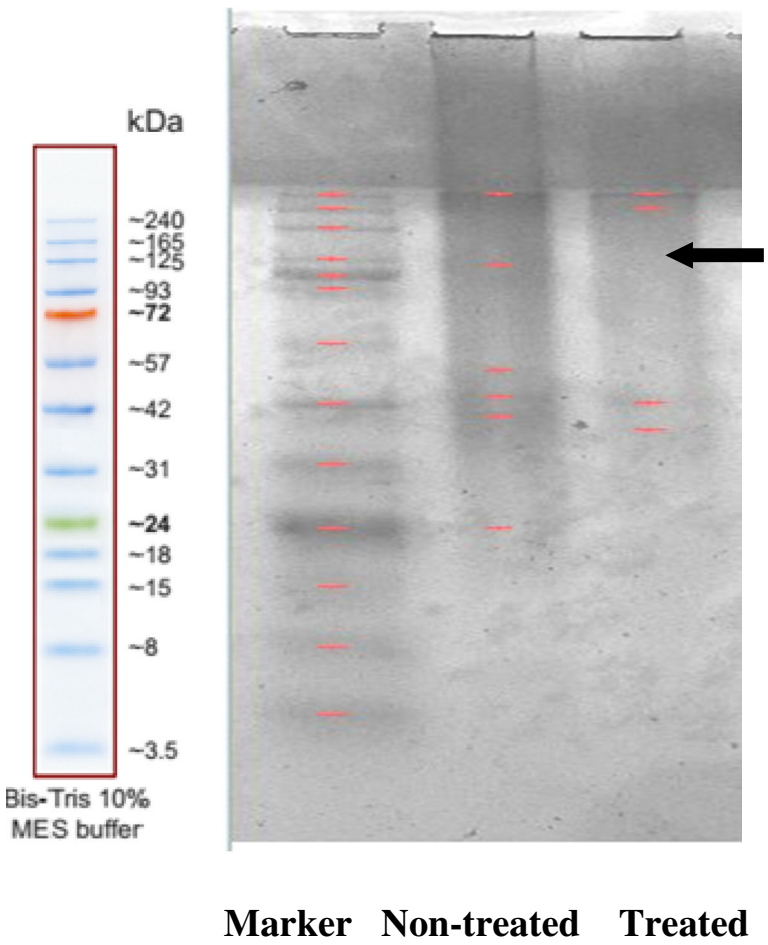

Fig. 2 Electrophoretic analysis showing molecular weight of antibacterial protein in *Bacillus sphaericus*-treated *Culex pipiens* mosquitoes

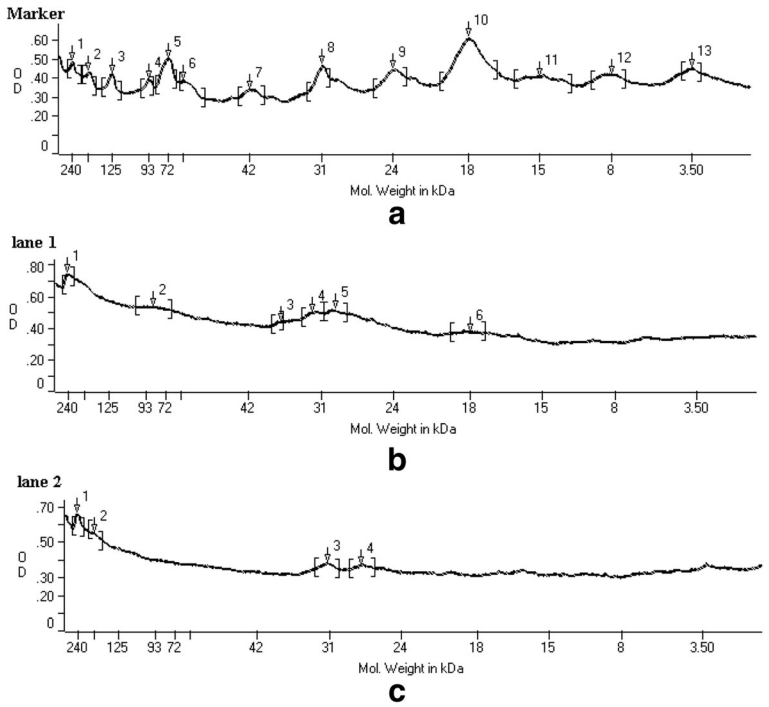

Fig. 3 Protein molecular weight and optical density of (**a**) marker, (**b**) non-treated, and (**c**) *Bacillus sphaericus*-treated *Culex pipiens* mosquitoes

microbial pathogens or released in the host during the infection (Pal and Wu, 2009). In addition to the signaling PRRs, insect genomes also contain secreted recognition molecules such as TEPs (Blandin and Levashina, 2004). Thioester-containing proteins are a major component of the innateimmune response of insects to invasion by bacteria and protozoa (Baxter et al., 2007). TEP1 is reported to be a secreted protein that circulates in the hemolymph of mosquitoes at all developmental stages starting from larval stage (Levashina et al., 2001). In the present study, secretion of the protein is most probably initiated in the larval stage, following bacterial treatment. Levashina et al. (2001) stated that TEP1 is produced by hemocytes and secreted from these cells as a full-length protein which is then cleaved in the hemolymph. This protein is a key immune factor that determines mosquito resistance to a wide range of pathogens (Pompon and Levashina, 2015). It has been identified as among the major mosquito factors that control parasite loads (Fraiture et al., 2009). In *Drosophila*, the TEP family is composed of six genes named Tep1-Tep6 (Bou Aoun et al., 2011).

In the present study, TEP was expressed in the hemolymph of adult mosquitoes emerged from bacteria-treated larvae. However, when bacteria were injected to adult stage, TEP was expressed in its proventriculus (Volohonsky et al., 2017). The latter authors reported also that shortly after the injection of bacteria, TEP1 can be detected inside the hemocytes that are attached to dissected mosquito carcasses.

The key feature of thioester-containing proteins is an internal β-cysteinyl-γ-glutamyl thioester bond (Janatova et al., 1980). The thioester is contained in a specific sequence motif, Cys-Gly-Glu-Gln (CGEQ), located in the loop before the first inner helix (α2) of the thioester domain (TED) (Fig. 4).

The mosquito TEPs have been found to possess conserved function, which results in the phagocytosis of bacteria (Shokal and Eleftherianos, 2017). In this study, this may lead to the emergence of resistance to bacteria in mosquitoes, especially when treated with high concentration.

Effector responses are initiated by damage signals such as reactive oxygen species signaling from epithelial cells and recognized by cell surface receptors on hemocytes (Baxter et al., 2017). Thioester-containing proteins (TEPs) are represented by multi-member families both in the fruitfly, *Drosophila melanogaster*, and in the mosquito, *Anopheles gambiae* (Blandin and Levashina, 2004). To date, there have been no reports of TEP detection in *Cx. pipiens*, confirmed in the present study.

The molecular weight of the protein at which its band was distinguished eliminates the probability of being defensins which are small (4.5 kDa) cationic/basic AMPs with six conserved cysteine residues (Ganz and Lehrer, 1994). Results of the present study hold opposing views from that of previous studies that propose the protein to be cecropin which is a protein of molecular weight around 4 kDa (Townson and Chaithong, 1991). The

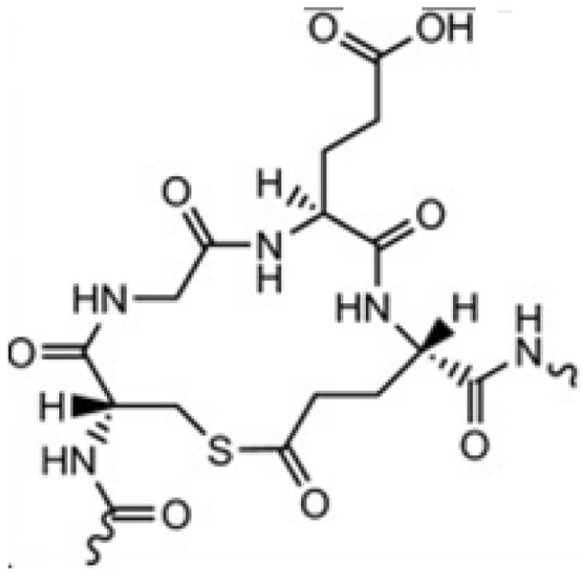

Fig. 4 TThioester domain (TED) (Khan et al., 1986)

present finding excludes the possibility of detecting any of glycine-rich family which is represented by 8–27-kDa proteins such as attacins (Kockum et al., 1984), sarcotoxins II (Ando and Natori, 1988), diptericin (Dimarcq et al., 1988), and coleoptericin (Bulet et al., 1991). Other excluded proteins also include proline-rich family which comprises small 2–4-kDa peptides mainly active against gram-negative bacteria and includes apidaecins (Casteels et al., 1989), abaecin (Casteels et al., 1990), drosocin (Bulet et al., 1993), and pyrrhocoricin (Cociancich et al., 1994).

The present study thus contradicts with that of Waterhouse et al. (2007) and Antonova et al. (2009) who concluded that the AMPs expressed in mosquitoes after immune challenge were mainly defensins and cecropins. Variation in the produced peptides may be due to variation of either the insect species or the bacterial strain. This conclusion agrees with that of Lowenberger (2001). Mosquito cecropins have a broad spectrum of antimicrobial activity (Lowenberger et al., 1999). The indication that antibacterial peptides are toxic to parasitic organisms has implications for their possible use in the disease vector control strategies of the future (Ham et al., 1994).

Earlier studies reported that immune activation by bacteria inoculation negatively influenced the development of *Plasmodium gallinaceum* and *P. berghei* in *Aedes* and *Anopheles* species (Lowenberger et al., 1999). In vivo transfection of AeTEP-1 into *Ae. aegypti* significantly reduced dengue virus infection (Cheng et al., 2011). Thioester-containing protein is known to be a central component in the innate immune response of *An. gambiae* to *Plasmodium* infection (Le et al., 2012, Volohonsky et al., 2017). However, no difference was seen between *Wuchereria bancrofti* development in *Escherichia coli* and *Micrococcus luteus*-inoculated *Cx. pipiens* mosquitoes as compared to non-inoculated controls (Bartholomay et al., 2003). These results, which are unlike those previously reported in model vector-parasite systems, could reflect either a variation in the immune capacity of the mosquito or the ability of the parasite to resist that response (Bartholomay et al., 2003).

Further studies are undergoing to elucidate the structural information of detected peptide.

Conclusions

A protein of 165 kDa was isolated in *B. sphaericus*-treated *Cx. pipiens* and characterized to be TEP1. Our findings suggest that this protein is involved in the antibacterial activity in mosquitoes. Understanding mosquito resistance mechanism to bacteria is crucial to build up programs to overcome such resistance.

Abbreviations

Ae : *Aedes*; *An* : *Anopheles*; *B*: *Bacillus*; *Cx*: *Culex*; *P*: *Plasmodium*; PAMPs: Pathogen-associated molecular patterns; PRRs: Pathogen recognition receptors; TED: Thioester domain; TEP1: Thioester-containing protein 1

Acknowledgements

The authors are thankful to the team of Genetic Engineering Research and Services Unit, Faculty of Science, Ain Shams University, for performing the electrophoresis. Gel analysis was carried out using Gel pro_Analyser program with the kind help of Dr. Shimaa E. Ibrahim, Faculty of Biotechnology, MUST University.

Funding

The research had no sources of funding.

Availability of data and materials

Additional data are available as additional file entitled "Gel Analysis."

Authors' contributions

MKT contributed to the design of the work, mosquito collection and rearing, bioassays and larval treatment with *B. sphaericus*, detection of antibacterial protein, preparing and revising the manuscript. WMS participated in the detection, commenting, and interpretation of antibacterial protein in bacteria-treated mosquitoes and prepared and revised the manuscript. BAS supervised the work; participated in the detection, commenting, and interpretation of antibacterial protein in bacteria-treated mosquitoes; and revised the manuscript. All authors read and approved the final manuscript.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

Publisher's Note

Author details

[1]Department of Zoology, Faculty of Science, Suez University, Suez, Egypt. [2]Department of Chemistry, Faculty of Science, Suez University, Suez, Egypt.

Received: 3 November 2017 Accepted: 23 February 2018
Published online: 29 March 2018

References

Ando K, Natori S (1988) Molecular cloning, sequencing, and characterization of cDNA for sarcotoxin IIA, an inducible anti-bacterial protein of *Sarcophaga peregrina* (flesh fly). Biochemist 27:1715–1721

Antonova Y, Alvarez KS, Kim YJ, Kokoza V, Raikhel AS (2009) The role of NF-kappaB factor REL2 in the *Aedes aegypti* immune response. Insect Biochem Mol Biol 39(4):303–314

Bartholomay LC, Farid HA, Ramzy RM, Christensen BM (2003) *Culex pipiens pipiens*: characterization of immune peptides and the influence of immune activation on development of *Wuchereria bancrofti*. Mol Biochem Parasitol 130(1):43–50

Baxter RH, Chang CI, Chelliah Y, Blandin S, Levashina EA, Deisenhofer J (2007) Structural basis for conserved complement factor-like function in the antimalarial protein TEP1. Proc Natl Acad Sci U S A 104(28):11615–11620

Baxter RH, Contet A, Krueger K (2017) Arthropod innate immune systems and vector-borne diseases. Biochemist 56(7):907–918

Blandin S, Levashina EA (2004) Thioester-containing proteins and insect immunity. Mol Immunol 40(12):903–908

Bou Aoun R, Hetru C, Troxler L, Doucet D, Ferrandon D, Matt N (2011) Analysis of thioester-containing proteins during the innate immune response of *Drosophila melanogaster*. J Innate Immun 3(1):52–64

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

Bulet, P., Dimarcq, J-L, Hetru, C., Lagueux, M., Charlet, M., Htgy, G., van Dorsselaer, A. and Hoffmann, J.A. 1993. A novel inducible antibacterial peptide of *Drosophila* carries an O-glycosylated substitution. J Biol Chem, 268: 14893-14897

Bulet P, Hetru C, Dimarcq JL, Hoffmann D (1999) Antimicrobial peptides in insects; structure and function. Dev Comp Immunol 23:329–344

Casteels P, Ampe C, Jacobs F, Vaeck M, Tempst P (1989) Apidaecins: antibacterial peptides from honeybees. EMBO J 8:2387–2391

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

Cheng G, Liu L, Wang P, Zhang Y, Zhao YO, Colpitts TM, Feitosa F, Anderson JF, Fikrig E (2011) An in vivo transfection approach elucidates a role for *Aedes aegypti* thioester-containing proteins in flaviviral infection. PLoS One 6(7):e22786

Cociancich S, Dupont A, Htgy G, Lanot R, Holder F, Hetru C, Hoffmann JA, Bulet P (1994) Novel inducible antibacterial peptides from a hemipteran insect, the sap sucking-bug *Pyrrhocoris apterus*. Biochem J 300:567–575

Dimarcq J-L, Keppi E, Dunbar B, Lambert J, Reichhart J-M, Hoffmann D, Rankine SM, Fothergill JE, Hoffmann JA (1988) Insect immunity. Purification and characterization of a family of novel inducible antibacterial proteins from immunized larvae of the dipteran *Phormia terranovae* and complete aminoacid sequence of the predominant member, diptericin A. Eul J Biochem 171:17–22

Fraiture M, Baxter RH, Steinert S, Chelliah Y, Frolet C, Quispe-Tintaya W, Hoffmann JA, Blandin SA, Levashina EA (2009) Two mosquito LRR proteins function as complement control factors in the TEP1-mediated killing of *Plasmodium*. Cell Host Microbe 5(3):273–284

Ganz T, Lehrer RI (1994) Defensins. Curr Opin Immunol 6:584–589

Ham PJ, Albuquerque C, Baxter AJ, Chalk R, Hagen HE (1994) Approaches to vector control: new and trusted. 1. Humeral immune responses in blackfly and mosquito vectors of filariae. Trans R Soc Trop Med Hyg 88(2):132–135

Harbach RE (2012) *Culex pipiens*: species versus species complex—taxonomic history and perspective. J Am Mosq Control Assoc 28(4Suppl):10–23

Harikrishna N, Rao MS, Murty US (2012) Immune peptides modelling of *Culex pipiens* sp by in silico methods. J Vector Borne Dis 49(1):19–22

Hill CA, Kafatos FC, Stansfield SK, Collins FH (2005) Arthropod-borne diseases: vector control in the genomics era. Nat Rev Microbiol 3(3):262–268

Janatova J, Lorenz PE, Schechter AN, Prahl JW, Tack BF (1980) Third component of human complement: appearance of a sulfhydryl group following chemical or enzymatic inactivation. Biochemist 19(19):4471–4478

Jayamani E, Rajamuthiah R, Larkins-Ford J, Fuchs BB, Conery AL, Vilcinskas A, Ausubel FM, Mylonakis E (2015) Insect-derived cecropins display activity against *Acinetobacter baumannii* in a whole-animal high-throughput *Caenorhabditis elegans* model. Antimicrob Agents Chemother 59(3):1728–1737

Kaushal A, Gupta K, Shah R, van Hoek ML (2016) Antimicrobial activity of mosquito cecropin peptides against Francisella. Dev Comp Immunol 63:171–180

Khan SA, Sekulski JM, Erickson BW (1986) Peptide models of protein metastable binding sites: competitive kinetics of isomerization and hydrolysis. Biochemist 25(18):5165–5171

Kockum K, Faye IV, Hofsten P, Lee J-Y, Xanthoponlos KG, Boman HG (1984) Insect immunity. Isolation and sequence of two cDNA clones corresponding to acidic and basic attacins from *Hyalophora cecropia*. EMBO J 3:2071–2075

Le BV, Williams M, Logarajah S, Baxter RH (2012) Molecular basis for genetic resistance of *Anopheles gambiae* to *Plasmodium*: structural analysis of TEP1 susceptible and resistant alleles. PLoS Pathog 8(10):e1002958

Levashina EA, Moita LF, Blandin S, Vriend G, Lagueux M, Kafatos FC (2001) Conserved role of a complement-like protein in phagocytosis revealed by dsRNA knockout in cultured cells of the mosquito, *Anopheles gambiae*. Cell 104:709–718

Lombardo F, Christophides GK (2016) Novel factors of *Anopheles gambiae* haemocyte immune response to *Plasmodium berghei* infection. Parasit Vectors 9:78–83

Lowenberger C (2001) Innate immune response of *Aedes aegypti*. Insect Biochem Mol Biol 31(3):219–292

Lowenberger CA, Bulet P, Charlet M, Hetru C, Hodgeman B, Christensen BM, Hoffmann JA (1995) Insect immunity: isolation of three novel inducible antibacterial defensins from the vector mosquito, *Aedes aegypti*. Insect Biochem Mol Biol 25(7):867–873

Lowenberger CA, Kamal S, Chiles J, Paskewitz S, Bulet P, Hoffmann JA, Christensen BM (1999) Mosquito–*Plasmodium* interactions in response to immune activation of the vector. Exp Parasitol 91(1):59–69

Mulla MS, Darwazeh HA, Tietze NS (1988) Efficacy of *Bacillus sphaericus* 2362 formulations against floodwater mosquitoes. J Am Mosq Control Assoc 4(2): 172–174

Paily KP, Agiesh Kumar B, Balaraman K (2007) Transferrin in the mosquito, *Culex quinquefasciatus* Say (Diptera: Culicidae), upregulated upon infection and development of the filarial parasite, *Wuchereria bancrofti* (Cobbold) (Spirurida: Onchocercidae). Parasitol Res 101:325–330

Pal S, Wu LP (2009) Pattern recognition receptors in the fly: lessons we can learn from the *Drosophila melanogaster* immune system. Fly 3(2):121–129

Pompon J, Levashina EA (2015) A new role of the mosquito complement-like cascade in male fertility in *Anopheles gambiae*. PLoS Biol 13(9):e1002255

Saugar JM, Rodríguez-Hernández MJ, de la Torre BG, Pachón-Ibañez ME, Fernández-Reyes M, Andreu D, Pachón J, Rivas L (2006) Activity of cecropin A-melittin hybrid peptides against colistin-resistant clinical strains of *Acinetobacter baumannii*: molecular basis for the differential mechanisms of action. Antimicrob Agents Chemother 50:1251–1256

Shokal U, Eleftherianos I (2017) Evolution and function of thioester-containing proteins and the complement system in the innate immune response. Front Immunol 8:1–9

Townson H, Chaithong U (1991) Mosquito host influences on development of filariae. Ann Trop Med Parasitol 85(1):149–163

Volohonsky G, Hopp AK, Saenger M, Soichot J, Scholze H, Boch J, Blandin SA, Marois E (2017) Transgenic expression of the anti-parasitic factor TEP1 in the malaria mosquito *Anopheles gambiae*. PLoS Pathog 13(1):e1006113

Waterhouse RM, Kriventseva EV, Meister S, Xi Z, Alvarez KS, Bartholomay LC, Mury CB, Bian G, Blandin S, Christensen BM, Dong Y, Jiang H, Kanost MR, Koutsos AC, Levashina EA, Li J, Ligoxygakis P, MacCallum RM, Mayhew GF, Mendes A, Michel K, Osta MA, Paskewitz S, Shin SW, Vlachou D, Wang L, Wei W, Zheng L, Zou Z, Severson DW, Raikhel AS, Kafatos FC, Dimopoulos G, Zdobnov EM, Christophides GK (2007) Evolutionary dynamics of immune-related genes and pathways in disease-vector mosquitoes. Science 316(5832):1738–1743