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A Comprehensive Review – Anatomical, Functional and Molecular profiling of honey bee wax moth Galleria mellonella

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***Abstract:*-- The greater wax moth Galleria mellonella attracts the view of many researchers by their anatomical, functional and molecular profiling patterns. Mostly the larvae feeds on honey bee hive and Larvae of the greater wax moth Galleria mellonella have been shown to provide a useful insight into the pathogenesis model of a wide range of microbial infections including mammalian fungal (Fusarium oxysporum, Aspergillus fumigatus, Candida albicans) and bacterial pathogens, such as Staphylococcus aureus, Proteus vulgaris, Serratia marcescens, Pseudomonas aeruginosa, Listeria monocytogenes or Enterococcus faecalis [1]. Like mammals, insects possess a complex innate immune system. In addition, analogies are found between the epithelial cells of insect larval midguts and intestinal cells of mammalian digestive systems. Finally, several basic components essential for the bacterial infection process such as cell adhesion, resistance to antimicrobial peptides, tissue degradation and adaptation to oxidative stress are likely to be important in both insects and mammals [2]. Thus, insects are polyvalent tools for the identification and characterization of microbial virulence factors involved in mammalian infections [1]. In 1934, many researchers focused on studying the larval body fluids to understand the biochemical mechanism of the honey bee moth. The larva of Galleria mellonella is available as in commercial for feeding the predatory insects. Not only this organism is used an innate immunity model but also it is now investigating for doing a research in genetic hereditary studies of sterility mechanism. The main aim of this review paper is to provide the current knowledge of this organism anatomical, functional, molecular profiling. My future aspects is to do whole genome sequence of this organism and also to create novel knock out model for understanding the interaction of foreign particles with the innate immune response disorders.**

***Keywords*: Galleria mellonella, mammalian fungal, bacterial pathogens, polyvalent tools, pathogenesis model, innate immunity model, sterility.**

I. INTRODUCTION

The Galleria mellonella bought a blessing from the bee keepers because this wax moth recycles combs of colonies that die in the wild as well as beeswax combs of honey hives in areas of dark, warm, and poorly ventilated. The larvae of this organism play a vital role in cause loss of honey hives of bee keepers and it is considered as the bee pest. On behalf of destruction these insects were excellent model for studying the immune system, inherited genetics, histopathology, pathogenicity, toxicology and host-pathogen interaction studies. The larvae does not digest the bee wax instead of they were lives on impurities in honey hives, it infects and starts to destroy the honey bees colonies. On annually the larvae cause huge loss for honey bee keepers. The greater wax moth is very common in south Indian and it belongs to the family Pyralidae. It is the only member of the genus Galleria. Greater wax moth (Galleria mellonella L.) larvae are used as suitable host for reproduction of bio-control agents like Entopathogenic nematode, Steinernema carpocapsae and natural enemies which includes Microplitis croceipes etc [3]. This wax worms shows excellent research interest for researchers for studying the pathogenicity, toxicology, innate immunity model and inherited sterility studies No stage can survive freezing temperatures. Adult moths fly to hives and lay eggs in crevices. The eggs hatch and the larvae burrow through the comb uncapping the honey and causing it to leak. Additionally the larvae lay silk as they burrow contaminating the honey.

Figure no: 1 the image of Galleria mellonella

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Taxonomy

The term wax moth is a common name which refers to different species of moths that invade, attack, and damage honeybee colonies and hive products [4]. This larvae fee on beeswax, dried apples, and other fruits, crude sugar, pollen, cast skins of larval bees, and dead insects. A pest of unprotected honey bee combs in weakened colonies or neglected hives. As the larvae chew through the comb, they spin a silk-lined tunnel through the cell walls and over the face of the comb. These silk threads can tether emerging bees by their abdomens to their cells and they die of starvation because they are unable to escape from their cell. This phenomenon is termed "galleriasis". The larvae are used as experimental subjects in insect physiology labs, as fish bait, and in the study of nematodes [5].

Kingdom: Animalia
Phylum: Arthropoda
Subphylum: Hexapoda
Class: Insecta
Order: Lepidoptera
Super family: Pyraloidea
Family: Pyralidae
Subfamily: Galleriinae
Tribe: Galleriini
Genus: Galleria
Species: mellonella – Greater wax moth
Binomial name: Galleria mellonella (Linnaeus 1758)
Anatomical profiling of Galleria mellonella

Egg:

Wax moth eggs vary in size, with an average length and width of 0.478 mm and 0.394 mm, respectively. The egg is of spheroidal shape with interspersed wavy lines which gives it a rough texture. The egg's colour varies from pink, through cream white to white, with little established knowledge on the mechanism(s) driving such colour changes [6].

Larva:

Upon hatching, wax moth larvae are approximately 1–3 mm in length and 0.12–0.15 mm in diameter. Prior to pupation, late instar larvae are about 25–30 mm in length and 5–7 mm in diameter. At the larval stage, sexing into male and female is not yet possible due to the absence of sex specific external morphological characters. The larva is polipod (eruciform), with six legs on the thorax and a number of prolegs on the third to sixth abdominal segments. The larva is cream white in colour, with its sclerotized body parts, although it darkens as it grows with each successful molt. The head is composed of three well developed apical teeth but lacks sub-apical teeth. It might be possible that the apical teeth confer an adaptive advantage and thus contribute to the destructive nature of larvae. Though not conspicuous with the naked eye, retractable antennae are present and visible under light microscopy [6].

Pupa:

The greater wax moth pupa averages 12–20 mm in length and 5–7 mm in diameter. Female pupae are normally longer than the males. The pupa is of obtect type, with all its extremities glued to the body by a secretion produced during ecdysis. At the onset of pupation, the pupa is white to yellow in colour, but gradually changes to brown and later to dark brown with age and development. Sexual dimorphism is present in the pupa just as in the adult stage. The female pupa possesses a cloven sternum which represents an aperture for the bursa copulatrix on its eighth abdominal segment (Figure 2a) while the male lacks this but instead possesses a small pair of external rounded knobs on the ventral side of the ninth abdominal segment which represent the phallomeres (Figure 2b). The pupa possesses a pair of prominent eyes and antennae are engrafted in mesowing. Pupation often takes place in spun cocoons covered with faecal pellets and frass and provided with an opening which serves as an exit for the eclosing adult [6].

Adult

The adult greater wax moth exhibits distinct sexual dimorphism. The female wax moth averages 15-20 mm in body length, 31 mm in wingspan, and 169 mg in weight. The male is considerably smaller and less dark in colour compared to the females. The forewings for both sexes show varying intensities of pigmentation with the anterior two-thirds covered by scales that give it a uniformly darker pigmentation. Larval diets and the developmental duration have been observed to influence adult body coloration. The female moth has an almost straight distal forewing margin as opposed to the scalloped (notched) wing margin in males' border. Additionally, the female possesses forward projecting labial palps which give the mouthparts a beak-like appearance ('pointed nose"), whereas in males it is curved sharply upwards and hooked inwards and appears snub-nosed. Despite their mouth parts, adult wax moths (both males and females) are not known to feed due to their rudimentary and bifurcated proboscis as opposed to the hollow tube-like proboscis in feeding lepidopteran adults. Both males and females have the same antenna type, filiform, which differ in their number of segments (40–50 in males and 50–60 in females). The greater wax moth has a typical six-part lepidopteran leg consisting of the coxa, trochanter, femur, tibia, tarsus, and pre-tarsus. Further, adult distinguishing features are found in the abdomen, where the ninth to eleventh segments have been modified into an adages and ovipositor in male and female moths, respectively [6].

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Life cycle

Adult moths are pale brown to grey, usually about 20 mm long. The grey wings are often mottled and appear as "roof" or "boat" shaped when folded over the body. Female moths usually lay 300 to 600 eggs in clusters on comb or in small cracks in hive material. The almost spherical, pinkish to white eggs are about 0.5 mm in diameter. The eggs hatch within 3 - 5 days when temperatures range from 29C to 35C. Hatching is delayed when temperatures are colder and at 18C hatching commences about 30 days after egg laying. Larvae are creamy white, but turn grey on reaching their fully grown size of up to 28 mm in length. After hatching, the small very active larvae tunnel in comb, lining their tunnels with silky web as they go. They move from comb to comb through a mass of webbing [7]. Newly hatched larvae may move to neighbouring honey bee colonies. Overseas laboratory studies have shown that newly hatched larvae can travel more than 50 metres. Wax moth larvae are very active in warm weather, but become inactive in the extreme cold of winter. At the optimum temperature of about 32C they reach full development about 19 days after hatching. At cooler temperatures, and when food is scarce, the larval period may extend to 5 months [7]. Fully developed larvae spin silky cocoons that may be found in a mass of webbing in the comb, and on the frames and internal surfaces of the hive. Larvae may form small canoe shaped depressions in the wooden hive components in which to spin their cocoons. Larvae can also bore through the wooden bars of frames. After spinning the cocoon, the larvae commence the pupal stage which lasts about 14 days when temperatures are high but as long as 2 months during cooler temperatures. After emergence, adult moths mate and the life-cycle begins again [7].

Functional profiling of Galleria mellonella

The following table represents the complete set of proteins with their genes name which are found by researchers and functions of the some were explained below followed by the table

Protein Names	Gene Names	Sequence status
Prophenoloxidase activating factor-like protein		Fragment
Fibroin heavy chain PG-2'		Fragment
Prophenoloxidase		Fragment
Period		Fragment
Polyubiquitin GmUblast		Fragment
Polyubiquitin GmUb1		Fragment
Period		Fragment
Apolipophorin		Fragment
Larval hemolymph protein		Fragment
Transferrin-like protein		Fragment
Gloverin-like protein		Fragment
Anionic antimicrobial peptide 2		Fragment
Inhibitor of apoptosis protein	IAP	Complete
RNA binding-like protein		Fragment
Cobatoxin-like protein		Complete
Heavy-chain fibroin	Fib-H	Fragment
Silk sericin	MG-3	Fragment
JH-inducible protein	9/27	Complete
Proline-rich protein	pro	Complete
Silk Sericin 1	MG-1	Complete
Silk Sericin 2	MG-2	Complete
Heavy-chain fibroin	Fib-H	Fragment
Caspase-1		Complete
Alpha-crystallin	alpha-crys/SHSP	Complete
Will die slowly	WDS	Fragment
Glutathione-S-transferase-like protein		Complete
Arylphorin		Complete
Prophenoloxidase		Complete
Hemolin		Complete
Yolk protein 2	Yp2	Fragment
Prophenoloxidase		Complete
Follicle-specific yolk polypeptide-4		Complete
Antifungal peptide gallerimycin		Complete
Cationic protein 8		Complete
Heat shock-like protein		Fragment
Flavin-binding storage protein		Fragment
Gallerin		Complete
NADH dehydrogenase subunit 6		Complete
Trypsin-like protein		Complete
Caspase-4	Casp-4	Fragment
Juvenile hormone binding protein		Complete
Putative enolase protein		Fragment
Silk protease inhibitor 1		Complete
Neuropeptide F	NPF	Complete
Caspase-1	Casp-1	Complete
Caspase-3	Casp-3	Complete
Caspase-6	Casp-6	Complete
Juvenile hormone binding protein	jhbp	Complete
Hexamerin	Lhp82	Complete
Peptidoglycan recognition-like protein A		Fragment
Tyrosine hydroxylase-like protein		Fragment
6tox		Complete
Ribosomal protein S7e		Complete
Crustacean cardioactive peptide	CCAP	Complete
Sericotropin		Complete
Beta-1,3-glucan recognition protein	bgrp1	Complete
Dopa decarboxylase		Fragment
Cell division cycle 2	cdc2	Complete
Autophagy related protein Atg6	ATG6	Complete
SGF1/FKH Homolog		Fragment
Kunitz-type protease inhibitor	KTPI	Complete
Moricin-like peptide C2	mor	Complete
Moricin-like peptide B	mor	Complete
Autophagy related protein Atg6	ATG8	Complete
Moricin-like peptide C4	mor	Complete
Moricin-like peptide C5	Mor	Complete
Moricin-like peptide A	Mor	Complete
Moricin-like peptide C1	Mor	Complete
Moricin-like peptide C3	Mor	Complete
Moricin-like peptide D	Mor	Complete
Carbamoylphosphate synthetase/aspartate transcarbamylase/dihydroorotase		Fragment
Silk protease inhibitor 2		Complete
Elongation factor 1-alpha	Ef-1a	Fragment
Cytochrome c oxidase subunit 2	COII	Fragment
Inducible serine protease inhibitor 3		Fragment
Cytochrome c oxidase subunit 3	COX3	Complete
Transferrin		Complete

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Calreticulin		Complete
Superoxide dismutase		Complete
Odorant receptor	Orco	Complete
ATP synthase subunit a	ATP6	Complete
Long wavelength sensitive opsin		Fragment
Protein Wnt		Fragment
Lipophorin receptor		Complete
Peptidoglycan recognition protein LB	pgrp-LB	Fragment
Peptidoglycan recognition-like protein B		Fragment
Interstitial collagenase	mmp1	Fragment
Cytochrome c oxidase subunit 1	COI	Fragment
NADH-ubiquinone oxidoreductase chain 2	ND2	Complete
Inducible serine protease inhibitor 2		Fragment
Pupal cuticle protein	PCP52	Complete
Lysozyme		Complete
Ferritin		Complete
Seroin		Complete
Sericin 1	SER 1	Fragment
Sericin 2	SER 2	Fragment
Cytochrome b	CYTB	Complete
NADH-ubiquinone oxidoreductase chain 3	ND3	Complete
NADH-ubiquinone oxidoreductase chain 5	ND5	Complete
NADH-ubiquinone oxidoreductase chain	ND4L	Complete
Cytochrome c oxidase subunit 2	COX2	Complete
ATP synthase protein 8	ATP8	Complete
NADH-ubiquinone oxidoreductase chain	ND4	Complete
NADH-ubiquinone oxidoreductase chain	ND1	Complete
Inducible serine protease inhibitor 1		Fragment
Apolipophorin-3		Complete
Inducible metalloproteinase inhibitor protein	IMPI	Complete
Defensin		Complete
Cecropin-D-like peptide		Complete
Defensin-like peptide		Complete
Ecdysone-inducible protein E75	E75	Complete
Lebocin-like anionic peptide 1		Complete
Proline-rich antimicrobial peptide 1		Complete
Fibrohexamerin	P25	Complete
Fibroin light chain	FIBL	Complete
Anionic antimicrobial peptide 2		Complete
Pro-corazonin	crz	Complete
Cytochrome c oxidase subunit 2	COII	Complete
Probable nuclear hormone receptor HR3	HR3	Complete
Proline-rich antimicrobial peptide 2		Complete
27 kDa hemolymph protein		Complete
Carotenoid isomerooxygenase	ninaB	Complete
Cytochrome c oxidase subunit 1	COI	Fragment

1. Apolipophorin-3 –

This protein belongs to the insect apolipophorin-3 family and equilibrium between a soluble monomer and a bound lipoprotein form. Apolipophorin-3 associates with lipophorin during lipid loading until each particle contains 9 or 14 molecules of apolipophorin-3. Assists in the loading of diacylglycerol, generated from triacylglycerol stores in the fat body through the action of adipokinetic hormone, into lipophorin, the hemolymph lipoprotein. It increases the lipid carrying capacity of lipophorin by covering the expanding hydrophobic surface resulting from diacylglycerol uptake. It thus plays a critical role in the transport of lipids during flight in several species of insects. Has antibacterial activity against the Gram-positive bacteria L.monocytogenes (MIC=6.5 µM).

It lacks antibacterial activity against the Gram-positive bacteria B.circulans, M.luteus, S.aureus, and S.lutea, and the Gram-negative bacteria E.coli D31, E.coli ATCC 25922, and S.typhimurium. It lacks antifungal activity against S.cerevisiae, P.pastoris, Z.marxianus, C.albicans, C.wickerhamii, A.niger, F.oxysporum, and T.harizianum. The Molecular mass is 5712.7 Da from positions 136 - 186 determined by ESI. The measured mass may be that of a peptide that is synthesized after immune challenge or a fragment of partial proteolytic digestion. [8].

2.Inducible metalloproteinase inhibitor protein – IMPI

The IMPI inhibits thermolysin, bacillolysin and pseudolysin, B.polymyxa metalloprotease and human MMP1 and MMP3. No activity on trypsin or cysteine protease papain. Five disulfide bonds are present. When artificially cleaved by thermolysin between Asn-56 and Ile-57, the two obtained chains (called heavy and light chains) remain linked. The N-terminus is blocked [9, 10]. Molecular mass is 8360 Da from positions 20 - 88 determined by MALDI.

3. Defensin

This protein belongs to the invertebrate defensin family Type 2 subfamily and has antifungal activity against A.niger (MIC=2.1 µM), C.albicans (MIC=8.5 µM), C.fructus (MIC=8.5 µM), F.oxysporum (MIC=16.9 µM), P.pastoris (MIC=16.9 µM), P.tannophilus (MIC=8.5 µM), T.harzianum (MIC=4.2 µM), and Z.marxianus (MIC=8.5 µM). Defensin lacks antibacterial activity against the Gram-positive bacteria L.monocytogenes, M.luteus, S.aureus, and S.lutea, and the Gram-negative bacteria E.coli D31, E.coli ATCC 25922, and S.typhimurium. Molecular mass is 4716.75 Da from positions 30 - 72 determined by MALDI. Defensin involves in the mechanism includes [8]:

- Defense response to fungus
- Innate immune response
- Killing of cells of other organism [8]

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4. Cecropin-D-like peptide
Cecropins have lytic and antibacterial activity against several Gram-positive and Gram-negative bacteria. It has antibacterial activity against the Gram-positive bacteria M.luteus (MIC=34.4 µM), L.monocytogenes (MIC=34.4 µM), and S.lutea (MIC=34.4 µM), and the Gram-negative bacterium E.coli D31 (MIC=8.6 µM). It lacks antibacterial activity against the Gram-positive bacterium B.circulans, and the Gram-negative bacteria E.coli ATCC 25922 and S.typhimurium. It has antifungal activity against A.niger, but lacks antifungal activity against C.albicans, C.wickerhamii, F.oxysporum, P.pastoris, P.tannophilus, S.cerevisiae, T.harzianum, and Z.marxianus [8].

5. Defensin-like peptide
It has antibacterial activity against the Gram-positive bacterium S.lutea (MIC=1.9 µM). It lacks antibacterial activity against the Gram-positive bacteria L.monocytogenes and M.luteus, and the Gram-negative bacteria E.coli D31, E.coli ATCC 25922, and S.typhimurium. It has antifungal activity against A.niger (MIC=2.9 µM), C.albicans (MIC=2.9 µM), C.fructus (MIC=2.9 µM), C.wickerhamii (MIC=2.9 µM), P.pastoris (MIC=2.9 µM), P.stiptis (MIC=2.9 µM), P.tannophilus (MIC=2.9 µM), T.harzianum (MIC=2.9 µM), and Z.marxianus (MIC=2.9 µM), but lacks antifungal activity against C.albidus, F.oxysporum, and S.cerevisiae [8].

6. Ecdysone-inducible protein (E75)
It belongs to the nuclear hormone receptor family (NR1 subfamily). Orphan receptor possibly involved in the regulation of genes in the ecdysteroid cascade. Regions include DNA binding and zinc finger. Both isoforms first expressed in stage-2 larvae and then highly expressed during larval and pupal molts. Only isoform E75A is expressed at the time of pupal ecdysis. Expression is induced by 20-OH-ecdysone which initiates and coordinates the molts [11].

7. Lebocin-like anionic peptide 1
It belongs to the lebocin family and antimicrobial protein. It has antibacterial activity against the Gram-positive bacteria M.luteus (MIC=22.7 µM) and L.monocytogenes (MIC=90.9 µM). It lacks antibacterial activity against the Gram-positive bacteria B.circulans, S.aureus, and S.lutea, and the Gram-negative bacteria E.coli D31, E.coli ATCC 25922, and S.typhimurium. It has antifungal activity against A.niger (MIC=90.9 µM) and T.harzianum (MIC=90.9 µM), but lacks antifungal activity against S.cerevisiae, P.pastoris, Z.marxianus, C.albicans, C.fructus, and F.oxysporum. The Molecular mass is 4820.1 Da from positions 1 - 42 and determined by ESI [8].

8. Proline-rich antimicrobial peptide 1
It is an anti-microbial protein and it has antibacterial activity against the Gram-positive bacterium M.luteus (MIC=55.0 µM). It lacks anti-bacterial activity against the Gram-positive bacteria B.circulans, and L.monocytogenes, and the Gram-negative bacteria E.coli D31 and E.coli ATCC 25922. It has antifungal activity against P.pastoris (MIC=16.5 µM), Z.marxianus (MIC=16.5 µM), S.pombe (MIC=11.0 µM), and C.wickerhami (MIC=16.5 µM), but lacks antifungal activity against A.niger and C.albidus. The Molecular mass is 4322 Da from positions 1 - 37 and determined by ESI [8].

9. Fibrohexamerin (P25)
Levels are low until the penultimate larval instar, and peak in feeding larvae of this instar. Expression decreases again at the molt to the last larval instar. Levels rise rapidly after ecdysis when the larvae initiate feeding and reach a maximum in slowly mobile pre-pupae at the end of cocoon spinning. There is then a ten-fold drop which coincides with the start of silk gland degeneration. Tissue specificity is produced exclusively in the posterior (PSG) section of silk glands, which are essentially modified salivary glands. Silk fibroin elementary unit consists in a disulfide-linked heavy and light chain and a p25 glycoprotein in molar ratios of 6:6:1. This results in a complex of approximately 2.3 MDa [12, 13, & 14].

10. Fibroin light chain (FIBL)
It is likely that the major role of L-chain is to prevent the retention of H-chain in ER by forming the disulfide linkage. Partially N-terminally processed to yield a short form which lacks the first two residues of the long form? The interchain disulfide bridge is essential for the intracellular transport and secretion of fibroin. Tissue specificity is produced exclusively in the posterior (PSG) section of silk glands, which are essentially modified salivary glands. The FIBL expressed in the posterior silk glands throughout the penultimate and last larval instars. Declines in immobile pupae and disappears within the next 12 hours when insects pupate. Silk fibroin elementary unit consists in a disulfide-linked heavy and light chain and a p25 glycoprotein in molar ratios of 6:6:1. This results in a complex of approximately 2.3 MDa. It has the Length is 267 with the Mass (Da):27,079.

11. Anionic antimicrobial peptide 2
It is an antimicrobial protein. Has antibacterial activity against the Gram-positive bacteria M.luteus (MIC=86.6 µM), L.monocytogenes (MIC=86.6 µM), and S.lutea

(MIC=86.6 µM). It lacks antibacterial activity against the Gram-positive bacteria B.circulans and S.aureus, and the Gram-negative bacteria E.coli D31, E.coli ATCC 25922, and S.typhimurium. Has antifungal activity against P.pastoris (MIC=86.6 µM) and P.stipitis (MIC=90.9 µM), but lacks antifungal activity against A.niger, C.albicans, C.albidus, C.fructus, C.wickerhamii, F.oxysporum, S.cerevisiae, S.pombe, T.harzianum, and Z.marxianus. Molecular mass is 6978.9 Da from positions 1 - 60 and it was determined by ESI. The tissue specificity of Anionic antimicrobial peptide 2 is the hemolymph [8].

12.Pro-corazonin (crz)

Cardioactive peptide Corazonin is probably involved in the physiological regulation of the heart beat. The tissue specificity is the four pairs of lateral neurosecretory cells in the brains of late instar larvae, pupae and adults. It crz has neuropeptide hormone activity. It has a length: 113 with the Mass (Da):12,766 [16].

13. Probable nuclear hormone receptor HR3 (HR3)

It is a Putative receptor whose ligand is not yet known. The expression of this receptor was by ecdysteroid. It has a length: 557 with the Mass (Da):62,316 [17].

14. Carotenoid isomerooxygenase (ninaB)

It catalyzes the oxidative cleavage at the 15, 15'-double bond of carotenoids and the simultaneous all-trans to 11-cis isomerization of one cleavage product. Carotenoids like 11-cis retinal can promote visual pigment biogenesis in the dark. It is an Essential for the biosynthesis of the 3-hydroxyretinal chromophore of rhodopsin from zeaxanthin and for proper photoreceptor development. The catalytic activity of ninaB is given below;

Zeaxanthin + O2 = (3R)-11-cis-3-hydroxyretinal + (3R)-all-Trans-3-hydroxyretinal

The cofactor of ninaB is fe2+ and this protein is involved in the pathway retinol metabolism, which is part of Cofactor metabolism. It has a length: 513 with the Mass (Da): 57,839 [18].

15. Capsid protein VP1

Capsid protein self-assembles to form an icosahedral capsid with a T=1 symmetry, about 22 nm in diameter, and consisting of 60 copies of size variants of the capsid proteins, which differ in the N-terminus capsid encapsulates the genomic ssDNA. Capsid proteins are responsible for the attachment to host cell receptors. This attachment induces virion internalization predominantly through clathrin-dependent endocytosis. The N-terminus of VP1 is sequestered within the mature capsid. It contains a phospholipase A2-like region and putative nuclear localization signals. It has 4 isoforms [19, 20].

16. Pupal cuticle protein PCP52 (PCP52)

It is a Component of the cuticle of the pupa of Galleria mellonella. Maximal expression during the first day after pupal ecdysis and Length: 353 Mass (Da): 35,818 [21].

17. Inducible serine protease inhibitor 1 and 2

It inhibits trypsin and the toxin protease PR2 of M.anisopliae. It does not inhibit chymotrypsin, subtilisin Carlsberg, proteinase K, porcine pancreatic elastase and the toxin protease PR1 of M.anisopliae. The developmental stage is Last instar larvae. Inducible serine protease inhibitor 1 and 2 has a length: 52 with mass (Da): 6,057 and Length: 50 Mass (Da):5,379 [22].

18. Sericin-1 (SER1) & Sericin-2 (SER2)

It provides the silk fibroin thread with a sticky coating. It acts as cement by sticking silk threads together. The tissue specificity is produced exclusively in the middle (MSG) section of silk glands. SER 1 has length: 115 with the Mass (Da): 9,161 and SER 2 has Length: 220 with the mass (Da): 20,301[23].

19. Transferrin

It belongs to the transferrin family. Transferrins are iron binding transport proteins which bind Fe3+ ion in association with the binding of an anion, usually bicarbonate. It has a length: 680 & mass (Da):75,779 [24].

20. Superoxide dismutase

It belongs to the iron/manganese superoxide dismutase family. It destroys radicals which are normally produced within the cells and which are toxic to biological systems. It has a Length: 216 with mass (Da): 24,018 [25].

21. Odorant receptor (Orco)

It belongs to the insect chemoreceptor super family and heteromeric odorant receptor channel (TC 1.A.69) family. The function of this receptor is unknown. It has Length: 474 with the Mass (Da):53,366 [26].

22. Protein Wnt

It belongs to the Wnt family and the Ligand for members of the frizzled family of seven transmembrane receptors. Length: 134 with the mass (Da): 15,049 [27, 28].

23. Caspase-4 (Casp-4)

It belongs to the peptidase C14A family and the function of casp-4 is unknown. The Length is 361 with the mass (Da):41,632 [29].

Molecular profiling of Galleria mellonella

1. Pathogenicity of Candida albicans isolates from bloodstream and mucosal candidiasis assessed in mice and Galleria mellonella. This study was to elucidate a possible association between the pathogenic potential of Candida albicans strains with a clinical entity, systemic versus superficial candidiasis. Specifically, we assessed the pathogenicity of two groups of clinical C. albicans isolates: isolates from bloodstream infection (S) versus isolates from vaginitis patients (M), in two experimental in vivo systems - mice and Galleria mellonella, in

comparison to a control strain (CBS 562). Mice and G. mellonella larvae were inoculated with CBS 562 and the different S and M isolates, and followed up for survival rate and survival time during 30 and 7 days, respectively. Candida kidney colonization of mice was assessed by histopathology and colony-forming units' enumeration. The results revealed: (1) S and M isolates had different behaviour patterns in the two models and varied in different parameters; (2) no statistically significant difference in pathogenicity between S and M isolates as whole groups was noted; (3) S14 was the most virulent isolate and close to the standard strain CBS 562 in both models. This study is distinctive in its outline combining two different groups of C. albicans clinical isolates originating from two different clinical entities that were assessed in vivo concurrently in two models [30].

2. G. mellonella as a model host for human pathogens has increased significantly in the last few years. Important studies were published from different countries for evaluating the pathogenesis of bacterial and fungal infections and for exploring the host defences against pathogens. Therefore, standardized conditions for the use of G. mellonella larvae need to be established. Recent research showed that the deprivation of G. mellonella larvae of food during the experiment caused a reduction in immune responses and an increased susceptibility to infection, suggesting that incubating of larvae in the presence or absence of nutrition may affect the results and comparisons among different laboratories [31].

3. Investigation of insect immune mechanisms provides important information concerning innate immunity, which in many aspects is conserved in animals. This is one of the reasons why insects serve as model organisms to study virulence mechanisms of human pathogens. From the evolutionary point of view, we also learn a lot about host-pathogen interaction and adaptation of organisms to conditions of life. Additionally, insect-derived antibacterial and antifungal peptides and proteins are considered for their potential to be applied as alternatives to antibiotics. While Drosophila melanogaster is used to study the genetic aspect of insect immunity, Galleria mellonella serves as a good model for biochemical research. Given the size of the insect, it is possible to obtain easily hemolymph and other tissues as a source of many immune-relevant polypeptides [32].

4. The high mortality rates and economic burden associated with fungal infections, plus the emergence of fungal strains resistant to antifungal drugs, make it necessary to get a deeper understanding of fungal pathogenesis, as well as to identify new target structures for antifungal drug development. Still, murine models are considered as the gold standard for studying pathogenesis, quantifying virulence, and analysing the efficacy of antifungal drugs. However, invertebrates, such as the larvae of the greater wax moth Galleria mellonella, are promising alternative hosts to address some of these questions, especially when a large number of fungal strains need to be evaluated. There is research in the utilization of the invertebrate model host G. mellonella, and compare the virulence potential of the most important human fungal pathogens, with the focus on different virulence potential of closely related species [33].

5. Humoral immune response of Galleria mellonella after repeated infection with Bacillus thuringiensis. The insect immune system relies on innate mechanisms only. However, there is an increasing number of data reporting that previous immune challenge with microbial elicitors or a low number of microorganisms can modulate susceptibility after subsequent lethal infection with the same or different pathogen. This phenomenon is called immune priming. Its biochemical and molecular mechanisms remain unravelled. Here we present that Galleria mellonella larva that survived infections induced by intrahemocelic injection of a low dose of Bacillus thuringiensis were more resistant to re-injection of a lethal dose of the same bacteria but not other bacteria and fungi tested. This correlated with enhanced activity detected in full hemolymph as well as in separated hemolymph polypeptides. In addition, we observed differences in the hemolymph protein pattern between primed and non-primed larvae after infection with the lethal dose of B. thuringiensis. Expression of genes encoding inducible defence molecules was not enhanced in the primed larvae after the infection with the lethal dose of B. thuringiensis. It is likely that priming affects the turnover of immune related hemolymph proteins; hence, upon repeated contact, the immune response may be more ergonomic [34].

6. A comparative study of three synthetic peptides, namely neutral Cecropin D-like G. mellonella (WT) and two cationic peptides derived from its sequence, ΔM1 (+5) and ΔM2 (+9) is reported in this work. The influence of charge on the interactions between peptides and membranes and its effect on phase were studied by calorimetric assays. Differential scanning calorimetry (DSC) showed that ΔM2 peptide showed the strongest effect when the membrane contained phosphatidylcholine (PC) and phosphatidylglycerol (PG), increasing

membrane fluidization. Fourier transform infrared spectroscopy (FTIR) was used to determine lipid segregation in the presence of peptides. When WT and ΔM1 bound to model membrane containing PG and PC (1:1 molar ratio) a separation of both lipids was observed. Meanwhile, ΔM2 peptide also induced a de-mixing of PG-peptide rich domains separated from PC. FTIR experiments also suggested that the presence of ΔM1 and ΔM2 peptides increased lipid carbonyl group hydration in DMPG membrane fluid phase; however, hydration at the interface level in fluid phase was notably increased in the presence of WT and ΔM1 peptides in DMPC/DMPG. Overall the increase in positively charged residues favours the interaction of the peptides with the negatively charged membrane and its perturbation [36].

7. The cellular immune response of Galleria mellonella larvae against three strains of the gram-negative bacterium Actinobacillus pleuropneumoniae: low-virulence (780), high-virulence (1022) and the serotype 8 reference strain (R8). Prohemocytes, plasmatocytes, granulocytes, oenocytoids and spherulocytes were distinguished according to their size and morphology, their molecular markers and dye-staining properties and their role in the immune response. Total hemocyte count, differential hemocyte count, lysosome activity, autophagic response, cell viability and caspase-3 activation were determined in circulating hemocytes of naive and infected larvae. The presence of the autophagosome protein LC3 A/B within the circulating hemocytes of G. mellonella was dependent on and related to the infecting A. pleuropneumoniae strain and duration of infection. Hemocytes treated with the high-virulence strain expressed higher levels of LC3 A/B, whereas treatment with the low-virulence strain induced lower expression levels of this protein in the cells. The apoptosis in circulating hemocytes of G. mellonella larvae after exposure to virulent bacterial strains occurred simultaneously with excessive cell death response induced by stress and subsequent caspase-3 activation [37].

8. Staphylococcus aureus is an opportunistic pathogen related to a variety of life-threatening infections but for which antimicrobial resistance is liming the treatment options. That myricetin, but not its glycosylated form, can remarkably decrease the production of several S. aureus virulence factors, including adhesion, biofilm formation, hemolysis and staphyloxanthin production, without interfering with growth. Myricetin affects both surface proteins and secreted proteins which indicate that its action is unrelated to inhibition of the agr quorum sensing system. Analysis of virulence related gene expression and computational simulations of pivotal proteins involved in pathogenesis demonstrate that myricetin downregulates the saeR global regulator and interacts with sortase A and α-hemolysin. Furthermore, Myr confers a significant degree of protection against staphylococcal infection in the Galleria mellonella model. The study reveals that the potential of Myr as an alternative multi-target anti-virulence candidate to control S. aureus pathogenicity [38].

II. FUTURE ASPECTS

1. After reviewing the reports of Galleria mellonella, I decided to do my future research to create a novel gene knock model of innate immunity. By this effort we can prevent the sacrifice of commercial laboratory animals such as rodents, guinea pigs, dogs, monkeys, and mice.
2. Another future aspect which is influenced to know about the whole genome sequence of Galleria mellonella. Instead of focusing the single genes interaction with host pathogen, by knowing the whole genome sequence, it's better to understand the exact pathogenicity, toxicity, immune response, which gene is responsible for the sensitivity and whether there may be a single or multiple gene action etc.
3. And also studying the olfactory and neuro system of Galleria mellonella will help the researchers to understand the mechanism hidden inside the greater wax moth.

III. DISCUSSIONS

Galleria mellonella, the greater wax moth attracts everyone interest on it, because many researchers have interested in understanding the many pathogens which are harmful to humans health. As usual everyone knows the insects have innate immune response, hence researchers were utilizing this mechanism against multi resistant strains of pathogens against host to understand the host-pathogen interactions. From this review, the information will cover the up-to date knowledge about this greater wax moth.

IV. CONFLICT OF INTEREST:

The authors declare no interest of conflicts.

REFERENCES

1. Ramarao N, Nielsen-Leroux C, Lereclus D. The Insect Galleria mellonella as a Powerful Infection Model to Investigate Bacterial Pathogenesis. Journal of Visualized Experiments : JoVE. 2012;(70):4392. doi:10.3791/4392.

2. Lemaitre B, Hoffmann J. The host defense of Drosophila melanogaster. Annu. Rev. Immunol. 2007;25:697–743. [PubMed]
3. Birah Ajanta, Chilana Poonam, Shukla Umesh Kumar, Gupta G.P. Mass rearing of greater wax moth (Galleria mellonella L.) on artificial diet. Indian Journal of Entomology: 2008 volume: 70, Issue: 4, ISSN: 0367-8288/ first page (389) last page (392).
4. Williams, J.L. Insects:Lepidoptera (moths). In Honey Bee Pests, Predators, and Diseases; Morse, R., Flottum, K., Eds.; AI Root Company: Medina, OH, USA, 1997; pp. 121–141.
5. Powell, J.A & P.A Opler 2009. Moths of Western North America. University of California Press, p.186.
6. Kwadha CA1,2, Ong'amo GO3, Ndegwa PN4, Raina SK5, Fombong AT6.Insects The Biology and Control of the Greater Wax Moth, Galleria mellonella. 2017 Jun 9;8(2). pii: E61. doi10.3390/insects8020061.
7. ISSN 1329-8062 Published and Authorised by: Department of Environment and Primary Industries 1 Spring Street Melbourne, Victoria
8. "Purification and characterization of eight peptides from Galleria mellonella immune hemolymph." Cytrynska M., Mak P., Zdybicka-Barabas A., Suder P., Jakubowicz T. Peptides 28:533-546(2007) [PubMed] [Europe PMC] [Abstract]
9. "Cloning and expression of an inhibitor of microbial metalloproteinases from insects contributing to innate immunity." Clermont A., Wedde M., Seitz V., Podsiadlowski L., Lenze D., Hummel M., Vilcinskas A. Biochem. J. 382:315-322(2004) [PubMed] [Europe PMC] [Abstract]
10. "Purification and characterization of an inducible metalloprotease inhibitor from the hemolymph of greater wax moth larvae, Galleria mellonella." Wedde M., Weise C., Kopacek P., Franke P., Vilcinskas A.
Eur. J. Biochem. 255:535-543(1998) [PubMed] [Europe PMC] [Abstract]
11. "Isolation, characterization and developmental expression of the ecdysteroid-induced E75 gene of the wax moth Galleria mellonella."
Jindra M., Sehnal F., Riddiford L.M. Eur. J. Biochem. 221:665-675(1994) [PubMed] [Europe PMC] [Abstract]
12. "The P25 component of Galleria silk." Zurovec M., Kodrik D., Yang C., Sehnal F., Scheller K. Mol. Gen. Genet. 257:264-270(1998) [PubMed] [Europe PMC] [Abstract]
13. "Characterization of the P25 silk gene and associated insertion elements in Galleria mellonella." Yang C., Teng X., Zurovec M., Scheller K., Sehnal F.
Gene 209:157-165(1998) [PubMed] [Europe PMC] [Abstract]
14. "Light-chain fibroin of Galleria mellonella L." Zurovec M., Vaskova M., Kodrik D., Sehnal F., Kumaran A.K. Mol. Gen. Genet. 247:1-6(1995) [PubMed] [Europe PMC] [Abstract]
15. "Light-chain fibroin of Galleria mellonella L." Zurovec M., Vaskova M., Kodrik D., Sehnal F., Kumaran A.K. Mol. Gen. Genet. 247:1-6(1995) [PubMed] [Europe PMC] [Abstract]
16. "Corazonin gene expression in the waxmoth Galleria mellonella." Hansen I.A., Sehnal F., Meyer S.R., Scheller K. Insect Mol. Biol. 10:341-346(2001) [PubMed] [Europe PMC] [Abstract]
17. "Isolation and developmental expression of the ecdysteroid-induced GHR3 gene of the wax moth Galleria mellonella." Jindra M., Sehnal F., Riddiford L.M.
Insect Biochem. Mol. Biol. 24:763-773(1994) [PubMed] [Europe PMC] [Abstract]
18. "NinaB combines carotenoid oxygenase and retinoid isomerase activity in a single polypeptide." Oberhauser V., Voolstra O., Bangert A., von Lintig J., Vogt K.
Proc. Natl. Acad. Sci. U.S.A. 105:19000-19005(2008)[PubMed] [Europe PMC] [Abstract]
19. "Organization and expression of the ambisense genome of densonucleosis virus of Galleria mellonella (GmDNV)." Tijssen P.
20. "The structure of an insect parvovirus (Galleria mellonella densovirus) at 3.7 A resolution." Simpson A.A., Chipman P.R., Baker T.S., Tijssen P., Rossmann M.G. Structure 6:1355-1367(1998) [PubMed] [Europe PMC] [Abstract]
21. "Expression cloning and characterization of a pupal cuticle protein cDNA of Galleria mellonella L." Kollberg U., Obermaier B., Hirsch H., Kelber G., Wolbert P.
Insect Biochem. Mol. Biol. 25:355-363(1995) [PubMed] [Europe PMC] [Abstract]
22. "Isolation and characterization of novel inducible serine protease inhibitors from larval hemolymph of the greater wax moth Galleria mellonella." Froebius A.C., Kanost M.R., Goetz P., Vilcinskas A. Eur. J. Biochem. 267:2046-2053(2000) [PubMed] [Europe PMC] [Abstract]
23. "Silk gland specific cDNAs from Galleria mellonella L." Zurovec M., Sehnal F., Scheller K., Kumaran A.K. Insect Biochem. Mol. Biol. 22:55-67(1992)
24. "Galleria mellonella transferrin precursor mRNA." Han J., Yun C.-Y. Submitted (JUL-2005) to the EMBL/GenBank/DDBJ databases
25. Kim Y., Kim H., Kim Y., Seo S. Submitted (MAY-2007) to the EMBL/GenBank/DDBJ databases cited for nucleotide sequences.
26. "Gene cloning and sequence analysis of the receptor gene Orco in Galleria mellonella L. (greater wax moth)."

Yang S. Submitted (JUN-2015) to the EMBL/GenBank/DDBJ databases
27. "A molecular phylogeny for the pyraloid moths (Lepidoptera: Pyraloidea) and its implications for higher-level classification." Regier J.C., Mitter C., Solis M.A., Hayden J.E., Landry B., Nuss M., Simonsen T.J., Yen S.-H., Zwick A., Cummings M.P. Syst. Entomol. 37:635-656(2012)
28. "Assembling the Tree of Life Lepidoptera." LepTree.net Regier J.C.
Submitted (MAR-2012) to the EMBL/GenBank/DDBJ databases
29. "A comprehensive characterization of the caspase gene family in insects from the order Lepidoptera." Courtiade J., Pauchet Y., Vogel H., Heckel D.G. BMC Genomics 12:357-357(2011) [PubMed] [Europe PMC] [Abstract]
30. J Mycol Med. 2016 Mar; 26(1):1-8. doi: 10.1016/j.mycmed.2015.12.006. Epub 2016 Feb 4. Pathogenicity of Candida albicans isolates from bloodstream and mucosal candidiasis assessed in mice and Galleria mellonella. Frenkel M1, Mandelblat M1, Alastruey-Izquierdo A2, Mendlovic S3, Semis R4, Segal E5.
31. Galleria mellonella as a model host for human pathogens: recent studies and new perspectives. Junqueira JC1. Virulence. 2012 Oct 1;3 (6):474-6. doi: 10.4161/viru.22493.
32. Immunity of the greater wax moth Galleria mellonella Wojda I1. Insect Sci. 2017 Jun; 24(3):342-357. doi: 10.1111/1744-7917.12325. Epub 2016 May 25.
33. Fungal Biol. 2016 Feb;120(2):288-95. doi: 10.1016/j.funbio.2015.06.002. Epub 2015 Jun 16. Galleria mellonella: An invertebrate model to study pathogenicity in correctly defined fungal species. Binder U1, Maurer E2, Lass-Flörl C2.
34. J Invertebr Pathol. 2017 Aug 10;149:87-96. doi: 10.1016/j.jip.2017.08.008. [Epub ahead of print] Humoral immune response of Galleria mellonella after repeated infection with Bacillus thuringiensis. Taszłow P1, Vertyporokh L1, Wojda I2.
35. Arch Biochem Biophys. 2017 Sep 1;629:54-62. doi: 10.1016/j.abb.2017.07.008. Epub 2017 Jul 15.
36. The increase in positively charged residues in cecropin D like Galleria mellonella favors its interaction with membrane models that imitate bacterial membranes. Oñate-Garzón J1, Ausili A2, Manrique-Moreno M1, Torrecillas A2, Aranda FJ2, Patiño E3, Gomez-Fernández JC4.
37. Cell Tissue Res. 2017 Jul 8. doi: 10.1007/s00441-017-2653-5. [Epub ahead of print] Differential cellular immune response of Galleria mellonella to Actinobacillus pleuropneumoniae. Arteaga Blanco LA1, Crispim JS2, Fernandes KM1, de Oliveira LL1, Pereira MF2, Bazzolli DMS2, Martins GF3.
38. Sci Rep. 2017 Jun 6;7(1):2823. doi: 10.1038/s41598-017-02712-1. Myricetin protects Galleria mellonella against Staphylococcus aureus infection and inhibits multiple virulence factors. Silva LN1,2, Da Hora GCA3, Soares TA3,4, Bojer MS5, Ingmer H5, Macedo AJ6,7, Trentin DS1,2,8.