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Note

Purification and partial characterization of a lectin from the midgut of the worker honeybee (*Apis mellifera*)

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Lectins are central components in the innate immune response of insects. They have been isolated from various insect tissues, including hemolymph, fat body, and gut, and their presence typically correlates with their specific immune or physiological functions. Although hemocyte-associated lectins, such as hemolectin (Hml), have been well characterized for their roles in clotting and encapsulation, the biological functions of midgut-derived lectins remain largely unknown in the honey bee (*Apis mellifera*). The midgut is one of the first lines of defence that regulates the molecular effectors of the immune system. Here, we report the isolation of a low-molecular-weight (~40 kDa) galactose/mannose-specific lectin from the midgut of the worker honey bee. Functional assays have shown that *Apis* lectin strongly binds to carbohydrate moieties, acts as a specific ligand for sugars, has a direct bacteriostatic effect *on E. coli*, and can induce bacterial aggregation. In particular, the melanization response in hemolymph was greatly upregulated after the treatment with lectin. This indicates the coordinating role of lectin in both cellular and humoral immunity. Our study provides the first evidence of functional activity for the gut-derived lectin in *A. mellifera*. These findings open new avenues for research on caste- and task-specific immune modulation, as foragers, nurse bees, and queens are subject to varying pathogen pressures.

Keywords: Lectins, immunity, honey bee, *Apis mellifera*, hemagglutination, antimicrobial activity

Introduction

Honeybees serve as a model of a social immune mechanism that reduces the transmission of pathogens within the colony[1,2]. They have developed a sophisticated innate immune system that helps to prevent diseases and maintain the health of their hive. Hemocytes that perform phagocytosis, encapsulation, and nodulation to eliminate pathogens and barriers like the exoskeleton and gut lining, which keep invaders out, are key components of their innate immunity[1,2].

Studies indicate the significant role of lectins, proteins with a carbohydrate recognition domain (CRD), a characteristic motif, such as Glu-Pro-Asn (EPN) motif or Gln-Pro-Asp (QPD) motif, in the innate immunity of insects as they recognize and bind to specific carbohydrate moieties on pathogen surfaces[1,3–6]. This binding assists them to opsonize pathogens, facilitates hemocyte engulfment through phagocytosis, or initiates signalling pathways that produce antimicrobial peptides[6]. Furthermore, certain lectins function similarly to the complement system of higher vertebrates by forming complexes that trigger responses to eradicate infections. *Drosophila* lectins such as DL1, DL2, and DL3 have been demonstrated to bind to bacteria and improve phagocytosis and encapsulation[7]. Similar agglutinating and antibacterial qualities are displayed by the lectins in *Manduca sexta*[8], *Chrysomya megacephala*[9], and *Musca domestica*[10]. Mei *et al.*[11] reported that the presence of Bm-lectin in *Bombyx mori*, a C-type lectin with CRDs, helps to identify microbial polysaccharides and stimulate the immune system. Conversely, certain lectins may facilitate pathogen infection, underscoring their multifaceted roles in insect immunity. In the mosquito *Aedes aegypti*, several forms of C-type lectins have been demonstrated to facilitate the entry of West Nile virus, dengue virus, and Japanese encephalitis virus by binding to viral outer proteins, enabling their attachment and entry into host cells[12–16]. Lectins have been shown to be involved in various functions in honeybee biology, such as development, immunity, and stress response. Hemolectin, a protein with a lectin-like domain, is present in a subpopulation of honeybee hemocytes and participates in hemolymph clotting[16]. *Apis cerana cerana* galectin AccGalectin1 functions in development, oxidative stress response, and immunity[17]. The control of certain lectin genes during development and microbial infection reveals their importance to honeybee immunity and biology[17,18]. Lectins have been reported to be expressed in the majority of tissue types of different organisms[5,20,21],

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yet, reports of expression in honey bees are limited, mostly to hemolymph analysis[16] or whole-genome level expression studies[18,21,22].

Considering the role of the midgut in immune responses and pathogen recognition[23–25], characterization of honey bee midgut-derived lectin could shed new light on their immune system. The present study focuses on the isolation of lectins in the midgut of the worker honeybee, *Apis mellifera*. This is the first report to explore midgut-derived lectins in honeybees and therefore provides a foundation for advancing other studies on how midgut lectins mediate the immune system and aspects of overall hive health.

Materials and Methods

Preparation of the midgut extract of worker honeybee

Healthy worker bees, *Apis mellifera*, were collected from the apiary of Krishi Vigyan Kendra, Baramati (18.14425°N latitude, 74.53068°E longitude) during October. Bees were further transported to the lab in ventilated carriers to avoid stress and ensure their normal health. The midguts of about 20–25 worker bees were aseptically dissected. Subsequently, a homogenate was obtained by homogenizing midguts in 5 mL of buffered insect saline (10 mM Tris/HCl, pH 7.4, 130 mM NaCl, 5 mM KCl, and 1 mM CaCl2)[26]. This homogenate was centrifuged at 10,000 × g for 10 minutes at 4°C to collect the supernatant, which contained the crude lectin. The supernatant was then further stored at -20°C until needed.

Purification of Honey Bee Lectin mAmlectin

Isolation of lectin from honey bee midgut extract (mAmlectin) was done as previously described[26]. Briefly, an equal volume of 10% suspension of trypsinized goat red blood cells (GRBCs) was added to a volume of approximately 50 mL of honey bee midgut extract, and incubated at 4°C for 1 h. Cells were resuspended in phosphate-buffered insect saline supplemented with 1 mM calcium chloride and washed until the absorbance of the supernatant was less than 0.005 at 280 nm (Agilent Cary 60). After that, the cells were resuspended in 10 mL of buffered insect saline containing either 2 mM EDTA or 200 mM galactose and incubated at 4°C to allow the dissociation of lectins. The cells were removed by centrifuging the suspension, and the clear supernatant was collected; the galactose-eluted lectin was dialyzed against insect saline for 12-16 hours to remove the galactose. Molecular weight of purified honey bee lectin with galactose affinity was determined using 10% SDS-PAGE. The proteins were revealed on staining with silver nitrate, and molecular weight was determined on comparison with a standard protein marker (Serva 6.5-200 kDa40)[27,28].

Hemagglutination and Hemagglutination Inhibition Assays

Hemagglutination and Hemagglutination Inhibition Assays were performed as described by Nabavi *et al*[29]. Two-fold serial dilutions of the lectin were prepared in V-bottom microtiter plates, and mixed with 2% suspension of trypsin-treated goat erythrocytes to perform hemagglutination assays. The plates were incubated at room temperature and assessed visually for hemagglutination. Different sugars, such as glucose, galactose, and mannose (Stock solution of 200 mM), were added to the lectin-RBC mixtures for the hemagglutination inhibition[30] tests and to determine the binding specificity of the lectin. The degree of inhibition was determined by observing the absence or reduction of hemagglutination.

Antimicrobial Activity of mAmlectin

The agar well diffusion method was employed to investigate the antimicrobial activity of purified lectin against *Escherichia coli* (*E. coli*). A suspension of *E. coli* was inoculated on nutrient agar plates. Subsequently, wells were created in the agar where all different concentrations of the lectin were added. Plates were incubated at 37 °C overnight, and zones of inhibition around each well were measured to assess the antimicrobial feature of the lectin. Phosphate-buffered saline (PBS) served as the negative control.

Melanization and Cell Aggregation Assays

A thorough analysis was conducted to determine the role of the bee midgut lectin in the immune pathways. Approximately 100 µg/mL of mAmlectin was used for melanization and bacterial cell aggregation assays. Melanization assay was performed based on the method of Yu *et al*.[30] with modifications to suit spectrophotometric analysis using cuvettes. Phenol oxidase activity was spectrophotometrically measured at 470 nm under various experimental conditions, i.e., mAmlectin, hemolymph and mAmlectin-hemolymph interaction, at different time points (5, 10, and 15 minutes). All

the assays were carried out thrice to replicate them (technical replicates). As well, independent experimental setups using honey bees were made to consider biological variability. To assess the capacity of lectin to aggregate *E. coli*, observations of bacterial cell clumping[31] were made using an optical microscope (Lynx). In the experiments, bovine serum albumin (BSA) served as the control.

Statistical Analysis

All data from experiments, such as antimicrobial activity, cell aggregation, and melanization, were collected in triplicate. For the phenol oxidase-based melanization assay, the statistical analysis was conducted based on Two-way analysis of variance ANOVA to determine the influence of sample type and time on the enzyme activity. Technical replicates were used to measure variability within measurements by the analysis.

Results

Extraction of mAmlectin from the midgut of the worker honey bee

The midgut is a critical site for nutrient absorption and pathogen interaction, making it a valuable target for studying immune proteins. Midgut tissues from healthy worker bees were aseptically dissected (Fig 1a and b) and subsequently homogenized to prepare for protein extraction. Affinity chromatography, based on the carbohydrate-binding specificity of lectins, as methodologically detailed by Haq *et al*.[26] was used to purify midgut-derived lectin (mAmlectin) from a crude extract. The purity of mAmlectin was assayed by the Lowry assay. The average yield of pure lectin from 5 midguts was 0.972 mg/mL. The molecular weight of the purified lectin was estimated to be approximately 40 kDa by SDS-PAGE analysis, as determined by comparison with a protein standard (Fig. 1c).

Haemagglutination and Sugar Inhibition Assays Confirm Lectin Specificity

The functional properties of purified mAmlect were further characterized through a hemagglutination assay, which evaluates its capacity to agglutinate red blood cells and determines carbohydrate-binding specificity. mAmlect agglutinated goat erythrocytes in the hemagglutination assay in direct proportion to its concentration. The haemagglutination activity was defined as 4 HU. The mAmlect specific activity was 0.372 HU/µg (or 372 HU/mg) and showed moderate agglutination capacity when compared to other

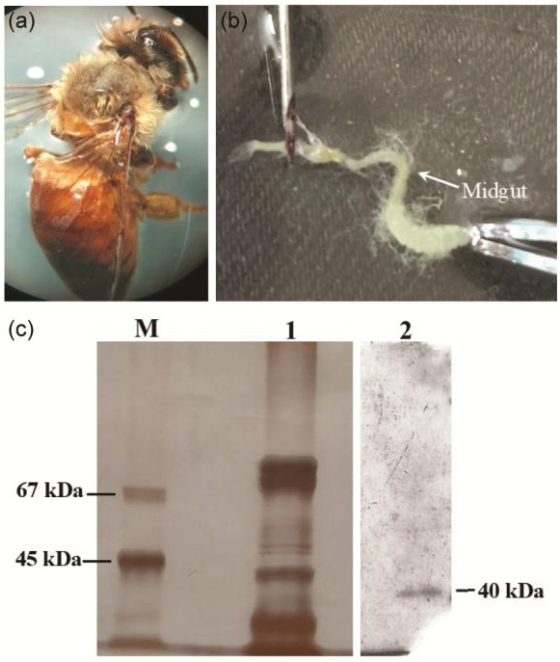

Fig. 1 — Purification of Lectin protein from the midgut of the worker honey bee: (a).Worker bee. (b) Digestive system of the worker bee, indicating the midgut region. (c) SDS PAGE analysis; lane M, Protein marker; lane 1, Mid gut protein extract; lane 2, Purified *Apis* lectin mAmlectin of 40 kDa.

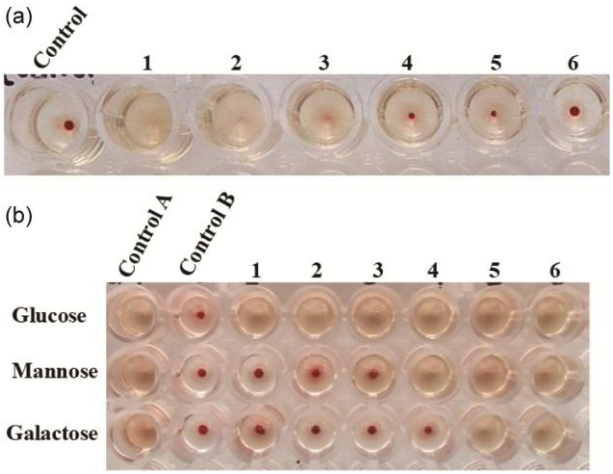

Fig. 2 — (a) Hemagglutination Assay of mAmlect showed agglutination. Control- RBCs in Phosphate buffered saline; Wells 1-6 mAmlectin + mAmlectin. (b) Hemagglutination inhibition assay of mAmlect performed using sugars as Glucose, Galactose, Mannose. Control A- lectin with RBCs, Control B- RBCs in Phosphate buffered saline, 1-6 wells of Row 1- glucose + mAmlectin + RBCs, Row 2- Galactose + mAmlectin + RBCs, Row 3- Mannose + mAmlectin + RBCs.

invertebrate lectins that have been documented (Fig. 2a). We performed hemagglutination inhibition assays using glucose, galactose, and mannose to study the specificity of the interaction. Mannose and galactose exhibited evident dose-dependent inhibition

of mAmlect-induced RBC agglutination, with up to 3 wells (MIC_{50} = 25 mM) inhibition for mannose and up to 4 wells (MIC_{50} = 50 mM) inhibition for galactose. However, glucose was unable to agglutinate even at the maximum tested concentration (200 mM), indicating that mAmlectin exhibits high binding specificity towards galactose, intermediate binding specificity towards mannose, and negligible binding specificity towards glucose (Fig. 2b). This carbohydrate-binding specificity of mAmlectin aligns with earlier findings from other insect species, including *Drosophila,* and demonstrates the vital function of lectins.

mAmlectin exhibits antimicrobial activity against *E. coli*

An antimicrobial activity assay was performed against *E. coli* to determine whether the mAmlectin can inhibit bacterial growth, suggesting a potential immune function beyond pathogen recognition. The mAmlectin displayed effective antimicrobial activity, as illustrated by the formation of clear inhibitory zones around the wells on an agar plate containing *E. coli* (Fig. 3). The inhibition zone diameters were also related to lectin concentration: the inhibition zone diameter increased with the concentration, thereby reinforcing the dose-dependent antimicrobial property of the lectin. In comparison, the wells containing only PBS, the negative control, showed no reduction in *E. coli* growth, providing further evidence to nullify the artifact of the testing procedure.

mAmlectin triggers innate immune responses in honeybees

The potential role of honey bee lectin in cell-mediated immunity was explored through *in vitro* melanization and bacterial cell aggregation assays. Hemolymph from worker bees was incubated with 100 µg/mL of mAmlectin in the presence of *E. coli* (1.6×10^5 cells/mL), and melanin production was monitored using absorbance at 470 nm over time. The absorbance was increased with time in hemolymph and lectin-hemolymph samples, and lectin alone revealed little change. Treatment with mAmlectin resulted in a significant increase in melanin production compared to control groups (hemolymph without mAmlectin and mAmlectin alone) (Fig. 4). Two-way ANOVA revealed significant effects of lectin on hemolymph melanization with time. It showed that sample type ($F(2, 45) = 100.553$, $P < 0.001$), time ($F(2, 45) = 38.504$, $P < 0.001$) and the interaction ($F(4, 45) = 8.103$, $P < 0.001$) had significant effects on melanization. The difference in

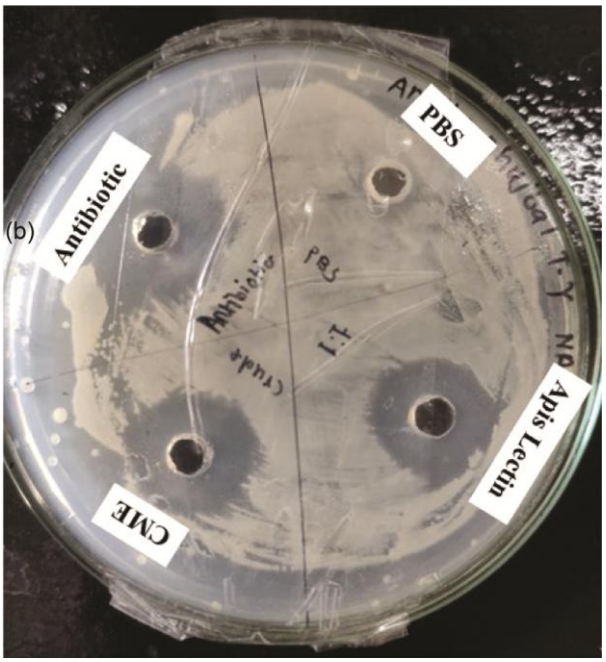

Fig. 3 — Agar well diffusion assay to show antibacterial activity of the mAmlectin against *E. coli*. Zones of inhibition were observed in samples, including crude midgut extract protein (CME) and mAmlectin (*Apis* lectin), which were comparable to the antibiotic control (Penicillin and Streptomycin solution, Himedia). Phosphate-buffered saline (PBS) was used as a negative control.

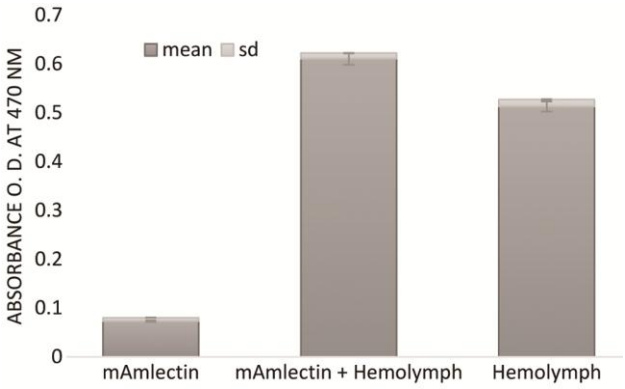

Fig. 4 — Enhancement of melanization due to mAmlectin. Measurement of hemolymph melanization with and without mAmlectin. Melanization was evaluated by measuring the absorbance at 470 nm at 10 mins. The data represent mean ± standard deviation from 3 independent experiments.

absorbance in hemolymph and hemolymph with mAmlectin was not statistically significant initially but significant at 10 minutes, indicating a time-dependent effect. The analysis implies that mAmlectin promotes melanin formation in honey bee hemolymph. The ability of mAmlectin to induce cell aggregation, a mechanism for immobilizing pathogens, was assessed *in vitro* by incubating 100

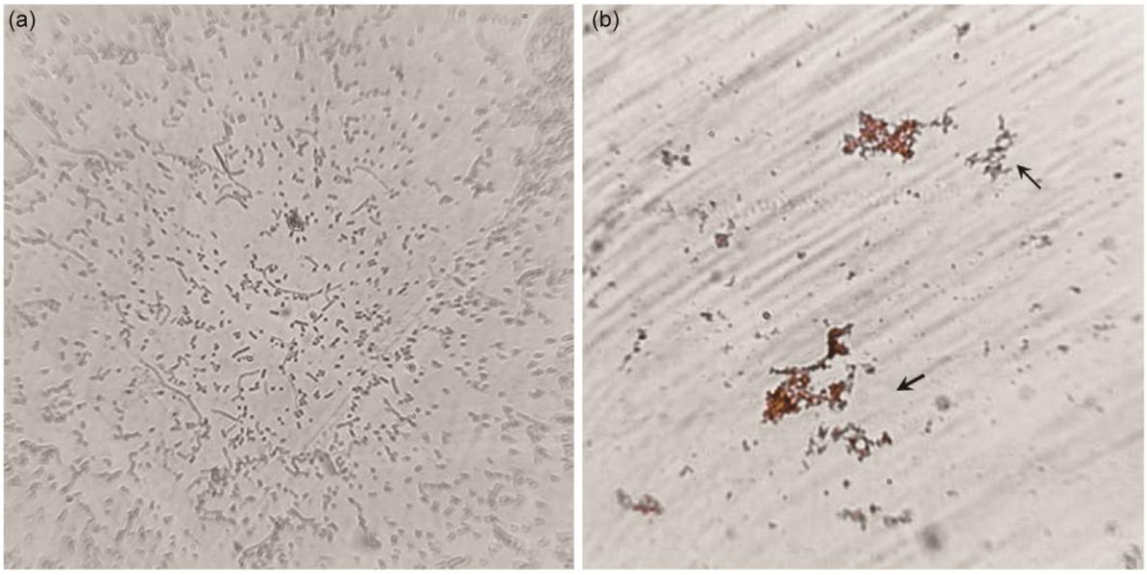

Fig. 5 — mAmlectin mediates the aggregation of *E. coli* cells: (a) *E. coli* cells treated with BSA showing no aggregation. (b) *E. coli* in the presence of mAMlectin- shows aggregation as indicated with arrows.

μg/ml of mAmlectin with *E. coli*. Microscopic observation revealed that mAmlectin induced aggregation of *E. coli* (Fig. 5a). In contrast, the control samples treated with BSA showed no aggregation of the *E. coli* cells (Fig. 5b), thus indicating that the bacterial clumping in this assay was solely mediated by the lectin and not by nonspecific protein interactions. This demonstrates the capacity of mAmlectin to facilitate the clumping of target cells.

Discussion

The present study provides a primary insight into the role of the midgut-derived lectin mAmlect, in the innate immunity of *A. mellifera* worker bees. The functional characterization of a mannose/galactose-specific lectin (≈approximately 40 kDa) from the honey bee midgut reveals its dual role in pathogen recognition, direct antimicrobial activity, and the activation of cellular immune responses. These results are consistent with earlier studies on insect lectins, concerning carbohydrate-binding specificity and haemagglutination activity[4,7,17,25,32,33]. Sugar-binding pattern and agglutination that are observed confirm that the mAmlect, similar to other insect lectins, could be playing an important role in innate immunity, i.e., pathogen detection and elimination through cell-mediated mechanisms. The midgut, an important barrier to ingested pathogens[34], underscores the promise of mAmlectin involvement in gut immunity. While most of the earlier studies in insects have focused on hemolymph-derived lectins[5], purification of mAmlectin from the midgut of the honey bee provides insight into compartmentalized immune functions. The single 40 kDa band of mAmlect observed on SDS-PAGE is similar to the subunit sizes as described for *Allomyrina dichotoma*[35] and *Drosophila melanogaster*[26]. This can be attributed either to a monomeric lectin or to a homomultimer with strongly interacting subunits. The molecular weight and electrophoretic mobility of mAmlect are thus in agreement with reported insect lectin patterns.

In addition to opsonization, the lectin exhibited direct antimicrobial activity against *E. coli*, which might involve membrane disruption via glycan cross-linking. Concomitantly, it boosted humoral immunity by enhancing melanization, a key process for sequestering and killing large numbers of pathogens. The ability of mAmlect to clump bacterial cells also supports a role in immobilizing the pathogens to the exposure of complement immune mechanisms, such as encapsulation[5,7,32,37]. This tag-aggregate-destroy mechanism is reminiscent of vertebrate complements[6] and is implemented in the context of insect innate immunity. Functionally, localisation of this lectin to the midgut indicates that it acts as a first defence against orally acquired pathogens that may, in turn, slow colony-level disease transmission by preventing the establishment and/or systemic spread of pathogens. Albeit *E. coli* offered a manageable model, testing with relevant bee pathogens (*Nosema ceranae*, *Melissococcus plutonius*) is necessary to

increase ecological validity. Furthermore, it is possible that environmental stressor agents that are commonly found in modern beekeeping could alter the expression or function levels of these lectins. Further studies should measure lectin activity in response to these stressors through transcriptomic and functional assay experiments using stressed bees. Inter-subspecific genetic differences could also generate lectin variants of varying potency which could be an informative dataset for a breeding programme. Finally, these *in vitro* results need to be tested at the level of the whole organism or colony since gut microbiota and social behaviors could modulate lectin function in the whole insect body.

Although hemolectin has been widely used to characterize the hemocytes in insects[37], and its orthologous gene, AmHml, has been disclosed in the genome of *Apis mellifera*[38,39], no gut-based lectins in honey bees have been previously investigated functionally. Recent studies, based on transcriptomic data, have shown the C-type lectins as key early response pattern recognition receptors in honeybee immunity[40]. Despite this, the findings primarily rely on correlations and lack strong biochemical support. In addition, vitellogenin, a multifunctional protein in the gut[41], involved in immune priming due to its pathogen-associated molecular pattern[42], might complicate the interpretation of gut-derived lectins. Amlectin reported in this study weighed approximately 40 kDa, making it much lower than the vitellogenin (~ 180 kDa). Moreover, the suggested galactose-dependent hemagglutination inhibition test promotes the carbohydrate-specific binding, which is a property of lectins[6]. On the contrary, vitellogenin tends to exhibit less specific pattern recognition. In view of this, complete characterization using advanced methods as mass spectrometry, is essential to properly identify and ascertain the role of the mid gut derived lectin in the honey bee. We propose that midgut lectins may act as early first-line recognition molecules and immune modulators at the gut surface of honeybees. This suggests a possible compartmentalization and specialization of lectins in honey bee immunity that deserves more attention.

Conclusion

This study offers evidence that midgut-derived lectin in *Apis mellifera* operates as a versatile immune effector of cellular and humoral immunity. While these findings offer us a partial insight, the gap remains about how environmental stressors, such as pesticides and climate variations, affect the expression and functionality of lectins. We hypothesize genetic diversity of lectin gene families among various bee species may be the reason for different immunity, which can be confirmed by the use of transcriptomics and genome-wide association studies. In essence, recognizing the implementation of the regulatory network as well as the evolutionary changes brought about by lectin-based immunity in honeybees will be instrumental in designing the strategic approaches to combat diseases responsible for increasing losses of the hive and the global decline of pollinators.

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Conflict of interest

The authors declare that they have no conflict of interest.

References

1 Schmid-Hempel P, Evolutionary ecology of insect immune defences, *Annu Rev Entomol*, 50 (2005) 529.

2 Hystad EM, Salmela H, Amdam GV & Münch D, Hemocyte-mediated phagocytosis differs between honey bee (Apis mellifera) worker castes, *PLoS One*, 12 (2017) e0184108.

3 Evans JD, Aronstein K, Chen YP, Imler JL, Kanost M, Thompson GJ, Zou Z &Hultmark D, Immune pathways and defence mechanisms in honey bees Apis mellifera, *Insect Mol Biol*, 15 (2006) 645.

4 Pees B, Yang W, Zárate-Potes A, Schulenburg H & Dierking K, High Innate Immune Specificity through Diversified C-Type Lectin-Like Domain Proteins in Invertebrates, *J Innate Immun*, 8 (2016) 129.

5 Xia X, You M, Rao XJ & Yu XQ, Insect C-type lectins in innate immunity, *Dev Comp Immunol*, 83 (2018) 70.

6 Fujita T, Matsushita M & Endo Y, The lectin-complement pathway - Its role in innate immunity and evolution. *Immunol Rev*, 198 (2004) 185.

7 Ao J, Ling E & Yu XQ, Drosophila C-type lectins enhance cellular encapsulation, *Mol Immunol*, 44 (2007) 2541.

8 Yu XQ, Gan H &Kanost MR, Immulectin, an inducible C-type lectin from an insect, Manduca sexta, stimulates activation of plasma prophenol oxidase. *Insect Biochem Mol Biol*. 29 (1999) 585.

9 Paulchamy R, Sreeramulu B, Karuppiah H, Arumugam G & Sundaram J, A serine protease-associated lectin in the cytolytic system of blowfly (Chrysomya megacephala)

larvae: Evidence and characterization, *Arch Insect Biochem Physiol*. 103 (2020) e21623.
10 Yu XQ &Kanost MR, Immulectin-2, a pattern recognition receptor that stimulates hemocyte encapsulation and melanization in the tobacco hornworm, Manduca sexta, *Dev Comp Immunol*, 28 (2004) 891.
11 Mei X, Li C, Peng P, Wang J, He E, Qiu Z & Shen D, Bombyx mori C-Type Lectin (BmIML-2) Inhibits the Proliferation of B. mori Nucleopolyhedrovirus (BmNPV) through Involvement in Apoptosis. *Int J Mol Sci.* 23 (2022) 8369.
12 Liu Y, Liu J, Pang X, Liu T, Ning Z & Cheng G, The roles of direct recognition by animal lectins in antiviral immunity and viral pathogenesis. *Molecules*. 20 (2015) 2272.
13 Liu K, Qian Y, Jung YS, Zhou B, Cao R, Shen T, Shao D, Wei J, Ma Z, Chen P, Zhu H & Qiu Y, mosGCTL-7, a C-Type Lectin Protein, Mediates Japanese Encephalitis Virus Infection in Mosquitoes, *J Virol*, 91 (2017) e01348.
14 Cheng G, Cox J, Wang P, Krishnan MN, Dai J, Qian F, Anderson JF &Fikrig E, A C-Type Lectin Collaborates with a CD45 Phosphatase Homolog to Facilitate West Nile Virus Infection of Mosquitoes, *Cell*, 142 (2010) 714.
15 Cheng L, Liu WL, Tsou YT, Li JC, Chien CH, Su MP, Liu KL, Huang YL, Wu SC, Tsai JJ, Hsieh SL & Chen CH, Transgenic Expression of Human C-Type Lectin Protein CLEC18A Reduces Dengue Virus Type 2 Infectivity in Aedes aegypti, *Front Immunol*, 12 (2021) 640367.
16 Gábor E, Cinege G, Csordás G, Török T, Folkl-Medzihradszky K, Darula Z, Andó I & Kurucz É, Hemolectin expression reveals functional heterogeneity in honey bee (Apis mellifera) hemocytes, *Dev Comp Immunol*, 76 (2017) 403.
17 Wang L, Wang C, Li H, Yang X, Wang Y, Guo X & Xu B, Isolation of AccGalectin1 from Apis cerana cerana and its functions in development and adverse stress response, *J Cell Biochem*. 120 (2019) 671.
18 Zou Z, Lopez DL, Kanost MR, Evans JD & Jiang H, Comparative analysis of serine protease-related genes in the honey bee genome: Possible involvement in embryonic development and innate immunity, *Insect Mol Biol*. 15 (2006) 603.
19 Bi J, Wang Y, Gao R, Liu P, Jiang Y, Gao L, Li B, Song Q & Ning M, Functional Analysis of a CTL-X-Type Lectin CTL16 in Development and Innate Immunity of Tribolium castaneum, *Int J Mol Sci*, 24 (2023) 10700.
20 Scott IM, Hatten G, Tuncer Y, Clarke VC, Jurcic K & Yeung KK-C, Proteomic analyses detect higher expression of c-type lectins in imidacloprid-resistant colorado potato beetle leptinotarsadecemlineata say, *Insects*,12 (2020) 3. doi: 10.3390/insects12010003.
21 Minozzi G, Lazzari B, De Iorio MG, Costa C, Carpana E, Crepaldi P, Rita R & Facchini E, Whole-Genome Sequence Analysis of Italian Honeybees (Apis mellifera), *Animals*, 11 (2021) 1311.
22 Sherbourne CC. *Lectins of the European Honey Bee (Apis Mellifera)*. La Trobe University, Master's thesis. OPAL Repository, latrobe.edu.au. 2023.
23 Lehane MJ, Wu D & Lehane SM, Midgut-specific immune molecules are produced by the blood-sucking insect Stomoxyscalcitrans, *Proc Natl Acad Sci USA*. 94 (1997) 11502.
24 Awais MM, Fei S, Xia J, Feng M & Sun J, Insights into midgut cell types and their crucial role in antiviral immunity in the lepidopteran model Bombyx mori, *Front Immunol*, 15 (2024)1349428.
25 Kumar V, Garg S, Sisodia D, Gupta L, Kumar S & Saxena V, Midgut immune profiling and functional characterization of Aedes aegypti ABC transporter gene(s) using systemic and local bacterial challenges, *ParasitVect*, 18 (2025) 34.
26 Haq S, Kubo T, Kurata S, Kobayashi A & Natori S, Purification, characterization, and cDNA cloning of a galactose-specific C-type lectin from Drosophila melanogaster. *J Biol Chem*. 271 (1996) 20213.
27 Sambrook J & Russell DW, SDS-Polyacrylamide Gel Electrophoresis of Proteins, *CSH Protoc*, 4 (2006). doi: 10.1101/pdb.prot4540
28 Bartsch H, Arndt C, Koristka S, Cartellieri M & Bachmann M, Silver staining techniques of polyacrylamide gels, *Methods Mol Biol*, 869 (2012) 481.
29 Nabavi Z, Baniardalani M &Basseri H, Purification and partial characterization of agglutinin lectin from heamolymph of german cockroach, blattella germanica, *J Arthropod Borne Dis*, 14 (2020) 144.
30 Yu XQ &Kanost MR, Immulectin-2, a lipopolysaccharide-specific lectin from an insect, Manduca sexta, is induced in response to Gram-negative bacteria, *J Biol Chem*, 275 (2000) 37373.
31 Liu J, Lou X, Yang Y, Zheng X, Zhang Y, Zhou P, Yang S, Huang M & Fei H, Functional characterisation and comparative analysis of two C-type lectins with different key motifs from mud crab, *Aquac Rep*, 25 (2022) 101208.
32 Ling E & Yu XQ, Cellular encapsulation and melanization are enhanced by immulectins, pattern recognition receptors from the tobacco hornworm Manduca sexta, *Dev Comp Immunol*, 30 (2006) 289.
33 Shen D, Tong M, Guo J, Mei X, Xia D, Qiu Z & Zhao Q, A Pattern Recognition Receptor C-type Lectin-S6 (CTL-S6) is Involved in the Immune Response in the Silkworm (Lepidoptera: Bombycidae), *J Insect Sci*, 21 (2021) 9.
34 Khan SA, Kojour MAM & Han YS, Recent trends in insect gut immunity, *Front Immunol*, 14 (2023) 1272143.
35 Umetsu K, Kosaka S & Suzuki T, Purification and characterization of a lectin from the beetle, Allomyrina dichotoma, *J Biochem*, 95 (1984) 239.
36 Lin Z, Wang JL, Cheng Y, Wang JX & Zou Z, Pattern recognition receptors from lepidopteran insects and their biological functions, *Dev Comp Immunol*, 108 (2020)103688.
37 Lesch C, Goto A, Lindgren M, Bidla G, Dushay MS &Theopold U, A role for Hemolectin in coagulation and immunity in Drosophila melanogaster, *Dev Comp Immunol*, 31 (2007) 1255.
38 Gábor E, Cinege G, Csordás G, Rusvai M, Honti V, Kolics B, Török T, Williams MJ, Kurucz É & Andó I, Identification of reference markers for characterizing honey bee (Apis mellifera) hemocyte classes, *Dev Comp Immunol*, 109 (2020) 103701.
39 Wallberg A, Han F, Wellhagen G, Dahle B, Kawata M, Haddad N, Simões ZL, Allsopp MH, Kandemir I, De la Rúa P, Pirk CW & Webster MT. A worldwide survey of genome sequence variation provides insight into the evolutionary

history of the honeybee Apis mellifera. *Nat Genet*. 46 (2014) 1081.

40 Lewkowski O, Poehlein A, Daniel R & Erler S, In the battle of the disease: a transcriptomic analysis of European foulbrood-diseased larvae of the Western honey bee (Apis mellifera), *BMC Genomics*, 23 (2022) 837.

41 Harwood G & Amdam G, Vitellogenin in the honey bee midgut, *Apidologie*, 52 (2021) 837.

42 Harwood G, Amdam G &Freitak D, The role of Vitellogenin in the transfer of immune elicitors from gut to hypopharyngeal glands in honey bees (*Apis mellifera*), *J Insect Physiol*, 112 (2019) 90.