Biological Control 212 (2026) 105944

Contents lists available at ScienceDirect

Biological Control

journal homepage: www.elsevier.com/locate/ybcon

Perspective

Functional characterization of four antimicrobial defensins in the globally invasive pest *Tuta* (*Phthorimaea*) *absoluta* (Lepidoptera: Gelechiidae)

Bo Xu [a], Guohui Zhang [b], Nannan Zhu [a], Jörg Romeis [c], Jana Collatz [c], Guifen Zhang [a], Cong Huang [a,*], Yibo Zhang [a,*], Fanghao Wan [a]

[a] State Key Laboratory for Biology of Plant Diseases and Insect Pests, Key Laboratory for Prevention and Control of Invasive Alien Species of Ministry of Agriculture and Rural Affairs, Institute of Plant Protection, Chinese Academy of Agricultural Sciences, Beijing 100193, China
[b] Engineering Research Center of Ecology and Agricultural Use of Wetland, Ministry of Education, Hubei Key Laboratory of Waterlogging Disaster and Agricultural Use of Wetland, College of Agriculture, Yangtze University, Jingzhou 434025, China
[c] Agroscope, Research Division Agroecology and Environment, Reckenholzstrasse 191, Zurich 8046, Switzerland

HIGHLIGHTS

- Four defensin genes (*tadef*1–4) identified in *Tuta absoluta*.
- The *tadef*1/2 are upregulated by both *Bacillus thuringiensis* and *Beauveria bassiana* infection.
- *Tadef*3/4 are upregulated by *B. thuringiensis* but suppressed by *B. bassiana*.
- *Tadef*1/2 peaked in early larvae and midgut.
- Recombinant tadef1/2 exhibited antibacterial activity against *B. thuringiensis*.

ARTICLE INFO

Keywords:
Tuta absoluta
Beauveria bassiana
Bacillus thuringiensis
Immune response
Biocontrol

ABSTRACT

Beauveria bassiana and *Bacillus thuringiensis* are widely utilized entomopathogens for biological control. Insects combat these microbial threats through the activation of innate immune defenses, with antimicrobial peptides (AMPs), particularly defensins, playing pivotal roles. *Tuta* (*Phthorimaea*) *absoluta* (Lepidoptera: Gelechiidae), is a rapidly spreading and highly destructive invasive tomato pest that has developed resistance to conventional insecticides. To enhance the efficacy of entomopathogens targeted by AMPs, we investigated defensin functions in *T. absoluta* during pathogen challenge. A genome-wide survey identified four defensin genes (*tadef1–4*), each encoding 76–124 amino acids (228–372bp ORFs). All contained the insect defensin motif (C-X_{5-16}-C-X_3-C-X_{9-10}-C-X_{4-7}-CX_1-C) and a conserved two-exon structure. Phylogenetic analysis confirmed their membership in the insect defensin family. Post-infection expression profiling showed that *tadef1/2* were upregulated by both *B. bassiana* and *B. thuringiensis*, while *tadef3/4* were suppressed by *B. bassiana* but induced by *B. thuringiensis* relative to uninfected controls. Developmental and tissue-specific expression analyses showed that *tadef1/2* peaked in early instar larvae, especially midgut. Recombinant tadef1/2 demonstrated antibacterial activity against *B. thuringiensis* but lacked antifungal effects against *B. bassiana*. These results establish tadef1/2 as key effectors against Gram-positive bacteria and provide a molecular basis for improving the biocontrol of *T. absoluta* via immune modulation.

1. Introduction

Insects, the most diverse taxonomic group on Earth, have developed a robust immune system as a result of continuous ecological pressures, which confers remarkable adaptability across diverse environments. In contrast to vertebrates that rely on adaptive immunity, insects depend exclusively on evolutionarily conserved innate immune mechanisms to detect and neutralize pathogens (Cytryńska et al., 2016; Hoffmann, 1995). Among these mechanisms, antimicrobial peptides (AMPs) function as primary humoral effectors capable of eliminating a wide range of

* Corresponding authors.
E-mail addresses: huangcong16@163.com (C. Huang), zhangyibo@caas.cn (Y. Zhang).

https://doi.org/10.1016/j.biocontrol.2025.105944
Received 11 September 2025; Received in revised form 18 November 2025; Accepted 9 December 2025
Available online 13 December 2025
1049-9644/© 2025 The Author(s). Published by Elsevier Inc. This is an open access article under the CC BY license (http://creativecommons.org/licenses/by/4.0/).

microbial threats, including Gram-positive and Gram-negative bacteria, fungi, and enveloped viruses, through membrane disruption or immunomodulatory pathways (Hanson et al., 2019; Wu et al., 2018). Structurally, insect AMPs are small (<10 kDa), peptides exhibiting broad-spectrum antimicrobial activity. These molecules are highly conserved and selectively target the membranes of prokaryotic cells or pathogenic eukaryotic cells via electrostatic interactions (Hoffmann, 1995; Bulet et al., 1999; Díaz-Garrido et al., 2021; Patyra and Kwiatek, 2023).

We focused on defensins, a major class of antimicrobial peptides (AMPs). These peptides are characterized by compact, disulfide-stabilized structures of 34–43 amino acids (Koehbach, 2017; Patyra and Kwiatek, 2023) and exhibit potent activity against Gram-positive bacteria, particularly *Bacillus thuringiensis* (Bt), a cornerstone biocontrol agent. Understanding the role of defensins in pathogen resistance is key to deciphering insect countermeasures and to designing effective resistance management strategies within integrated pest management (IPM) programs (Ganz and Lehrer, 1994; Tonk et al., 2015). Originally identified as sapecins in *Phormia terranovae* (Diptera: Calliphoridae) and *Sarcophaga peregrina* (Diptera: Sarcophagidae) (Lambert et al., 1989; Matsuyama and Natori, 1988), insect defensins were subsequently found in Lepidoptera species such as *Heliothis virescens* (Lamberty et al., 1999), *Spodoptera frugiperda* (Lepidoptera: Noctuidae) (Volkoff et al., 2003), *Bombyx mori* (Lepidoptera: Bombycidae) (Kaneko et al., 2008), and *Manduca sexta* (Lepidoptera: Sphingidae) (He et al., 2015). To date, more than 170 defensins have been identified and characterized in invertebrates (Patyra and Kwiatek, 2023). In general, insect defensins demonstrate potent antimicrobial activity against Gram-positive bacteria, including *B. thuringiensis*, *B. subtilis*, *Staphylococcus aureus*, *B. megaterium*, and *Micrococcus luteus*. Some defensin variants also exhibit activity against Gram-negative bacteria (*Escherichia coli*) and fungi, thereby broadening their antimicrobial spectrum (Jo et al., 2021; Thevissen et al., 2004; Xu et al., 2016).

The production of insect defensins is predominantly governed by the Toll and Imd (Immune deficiency) signaling pathways, which are activated upon recognition of pathogen-associated molecular patterns (PAMPs) (Ali Mohammadie Kojour et al., 2020; Lemaitre, 2004; Leulier and Lemaitre, 2008; Zhao et al., 2023a,b). Activation of these pathways triggers the nuclear translocation of NF-κB transcription factors—Dorsal/Dif in the Toll pathway and Relish in the Imd pathway—which subsequently initiate defensin gene transcription (Matova and Anderson, 2006; Tanji et al., 2010). After translation, the resulting pre-propeptides undergo proteolytic processing to yield mature defensins, which are secreted into the hemolymph to mediate systemic antimicrobial defense.

The South American tomato pinworm, *Tuta absoluta* (Lepidoptera: Gelechiidae), also known as *Phthorimaea absoluta*, is a major pest of tomato (*Solanum lycopersicum*) with devastating economic consequences (Biondi et al., 2018; Desneux et al., 2010; Guedes and Picanço, 2012). In the absence of effective control, it can cause annual yield losses in tomato crops ranging from 80 % to 100 % (Desneux et al., 2010). Conventional management strategies have primarily depended on chemical insecticides, including avermectins (Guedes et al., 2019), spinosad (Campos et al., 2014), and diamides (Silva et al., 2016). However, widespread resistance to these compounds has developed in key tomato-producing regions such as Chile, Brazil, Argentina, and China (Campos et al., 2014, 2014; Guedes et al., 2019; Zhang et al., 2025). Compounding the challenge, its polyphagy and endophytic larval feeding behavior significantly reduce the effectiveness of pesticide applications (Wang et al., 2024). As a result, sustainable alternatives, such as biological control, are urgently required. Recent studies have demonstrated the potential of modulating insect immune responses to enhance the efficacy of biocontrol agents (Zhao et al., 2023a,b). Nevertheless, our understanding of the immune system in *T. absoluta*, particularly at the molecular level in terms of defense mechanisms against microbial pathogens, remains limited.

In this study, we conducted a genome-wide identification of defensin genes in *T. absoluta* and designated them as *tadef1* through *tadef4*. We investigated their expression profiles following exposure to *B. bassiana* and *B. thuringiensis*, and functionally characterized *tadef1* and *tadef2* through tissue-specific and developmental expression analyses, *in vitro* recombinant protein expression, and antimicrobial activity assays. Our findings elucidate the distinct roles of these defensins in the immune response of *T. absoluta*, offering potential molecular targets for enhancing the efficacy of entomopathogens in integrated pest management strategies.

2. Materials and methods

2.1. Insects, fungi, and bacteria

T. absoluta larvae were collected from Lingqiu City, Shanxi Province, China. The insect population was reared on *S. lycopersicum cv*. Dafen (Shandong Shouhe Seed Industry Co. Ltd., Jinan, China) under controlled laboratory conditions at the Institute of Plant Protection, Chinese Academy of Agricultural Sciences (IPPCAAS), Beijing. The rearing environment was maintained at 26 ± 1 °C, with a 14:10-hour light/dark photoperiod and a relative humidity of 65 ± 10 %.

B. bassiana (strain Bb1Bm), preserved in the China General Microbiological Culture Collection (CGMCC No. 41260), was kindly provided by Professor Zhou Yi from Yangtze University, Jingzhou, China. Commercial *B. thuringiensis* (BTA) powder was obtained from GaoJiaoYan (Beijing) Technology Co., Ltd.

2.2. Defensin gene identification, structure, and chromosomal mapping

Protein sequences of insect defensins (Table S1) were obtained from NCBI (https://www.ncbi.nlm.nih.gov/) and used as queries in iterative BLASTP searches against the *T. absoluta* genome using TBtools (Chen et al., 2023), applying an E-value threshold of 1×10^{-5}. Conserved domains were predicted using NCBI-CDD (https://www.ncbi.nlm.nih.gov/cdd/) and InterPro (https://www.ebi.ac.uk/interpro/) (Blum et al., 2025). Final validation was carried out through NCBI BLASTP analysis. Gene structure and chromosomal locations were visualized with GSDS v2.0 (https://gsds.gao-lab.org/).

2.3. Phylogenetic analysis

To investigate the evolutionary relationships of *T. absoluta* defensins, 43 defensin sequences from 40 species (Table S1), representing insects, arachnids, and crustaceans, were selected for phylogenetic analysis. A neighbor-joining tree was generated using MEGA X with 1000 bootstrap replicates. Tree visualization and annotation were performed using iTOL v7.0 (https://itol.embl.de/).

2.4. Pathogen-induced expression of tadef genes

B. bassiana was cultured on Sabouraud Dextrose Agar supplemented with Yeast Extract (SDAY) for 14 days. Conidia were collected into 0.1 % Tween-80 and adjusted to a concentration of 1×10^5 conidia/mL. Uniform second-instar larvae were immersed in the suspension for 10 s, air-dried on sterile filter paper, and then transferred to 15-cm Petri dishes containing tomato leaves. Larvae were collected at 1, 3, 5, and 7 days post-inoculation (dpi) for RNA extraction. Control larvae were treated with 0.1 % Tween-80 alone. Each treatment consisted of four biological replicates, with 10 larvae per replicate ($n = 40$).

B. thuringiensis powder was dissolved in ddH_2O to a final

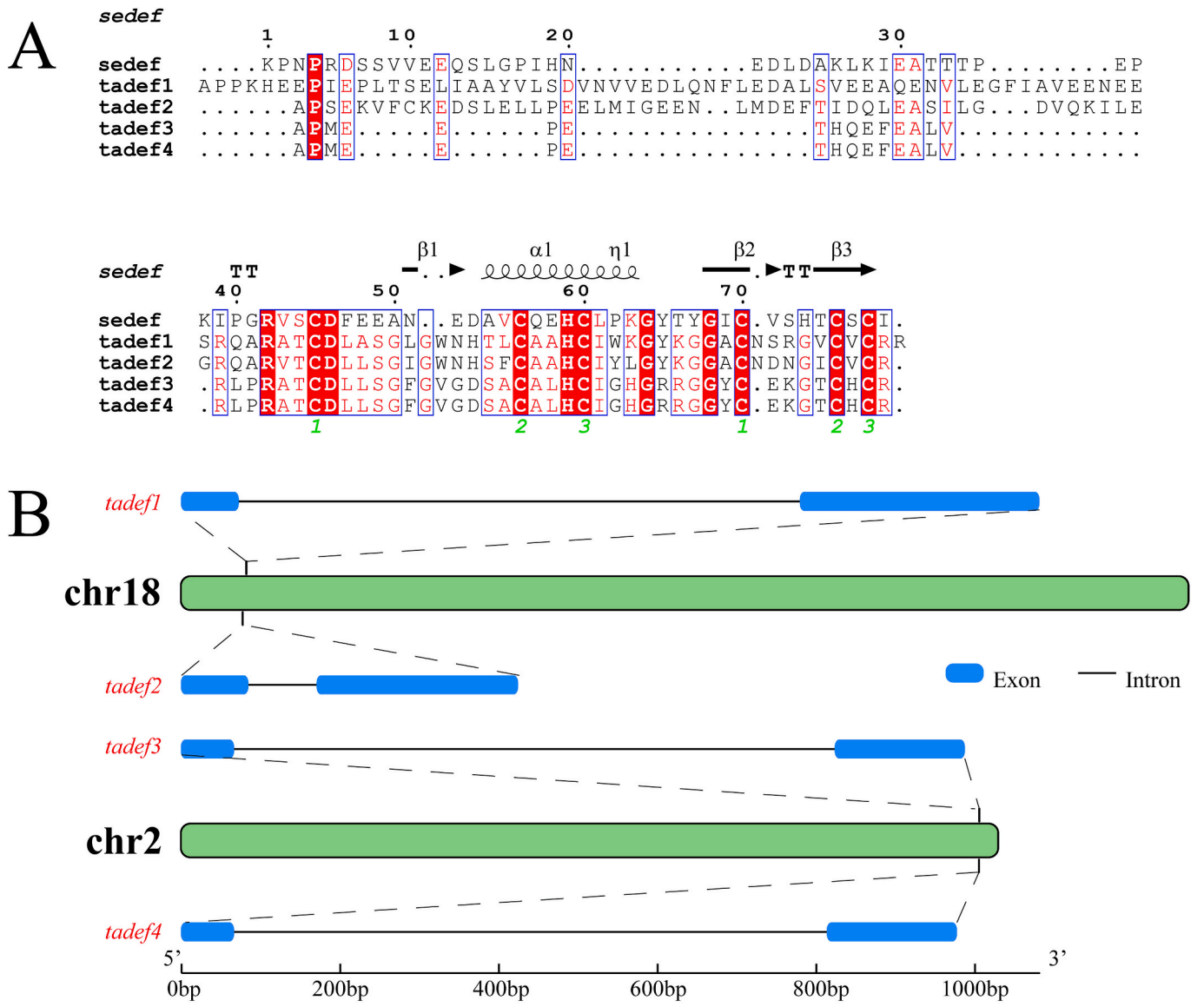


Fig. 1. Sequence alignment, gene structure, and chromosomal mapping of *tadefs*. (A) Multiple sequence alignment of *T. absoluta* defensins (tadef1–4) with homologous insect defensins, highlighting six conserved cysteine residues (green) critical for disulfide bond formation and structural stability. (B) Genomic organization and chromosomal locations of *tadefs* in *T. absoluta*, with exon–intron structure indicated (boxes = exons; lines = introns). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

concentration of 5 g/L. A 500 mL bacterial suspension was prepared, and tomato leaves were immersed in the solution for 10 s and then air-dried. Uniform second-instar larvae were placed on treated leaves within Petri dishes, with leaf petioles wrapped in moist cotton to maintain freshness. Larvae were collected at 12, 24, 48, and 72 h post-treatment for analysis. Control groups were exposed to leaves treated with ddH_2O only. Each treatment consisted of four biological replicates, with 10 larvae per replicate ($n = 40$).

Total RNA was extracted using the Total RNA Extraction Micro Kit (Genstone Biotech, Beijing, China), and cDNA synthesis was carried out with the Hifair® III 1st Strand cDNA Synthesis SuperMix for qPCR (Yeasen Biotechnology, Shanghai, China). The cDNA was diluted at a ratio of 1:4 and used as a template (0.5 μL) in RT-qPCR assays conducted on an ABI 7500 Real-Time PCR System using Hieff™ qPCR SYBR® Green Master Mix (Low Rox Plus) (Yeasen). Each reaction was performed in a total volum of 10 μL under the following conditions: initial denaturation at 95 °C for 5 min, followed by 40 cycles of 95 °C for 10 s and 60 °C for 34 s, concluding with melting curve analysis. All reactions were run in technical duplicates. The relative expression levels of tadef genes was calculated using the $2^{-\Delta\Delta CT}$ method and normalized to the reference gene ribosomal protein L5 (*RPL5*). Primer sequences are provided in Table S2.

2.5. Developmental, sex-specific, and tissue-specific expression of tadef1 and tadef2

Samples were collected from eight developmental stages: 120 first-instar larvae, 120 second-instar larvae, 80 third-instar larvae, 40 fourth-instar larvae, 40 male pupae, 40 female pupae, 40 male adults, and 40 female adults (total $n = 520$). For tissue-specific expression profiling, fat body, epidermis, midgut, and malpighian tubules were dissected from 120 second-instar larvae for each tissue type. All samples were uninfected and immediately flash-frozen in liquid nitrogen and stored at −80 °C prior to RNA extraction. RNA extraction, cDNA synthesis, and RT-qPCR were conducted as described in Section 2.4.

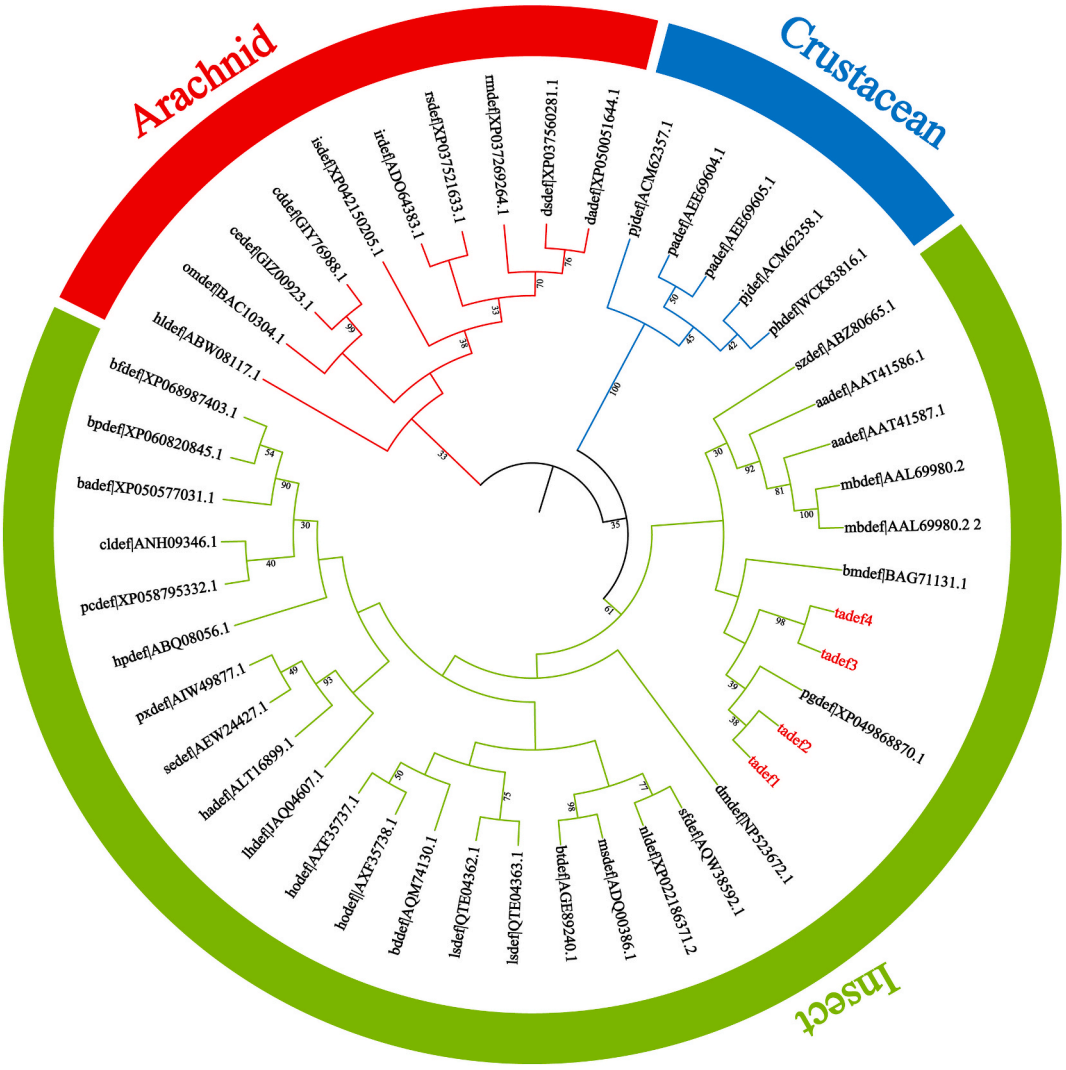


Fig. 2. Phylogenetic tree based on amino acid sequences of defensins from *T. absoluta* and other organisms.

2.6. Expression and purification of the recombinant protein tadef1 and tadef2

RNA extraction and cDNA synthesis were carried out according to the protocols described in Section 2.5. Primers were designed using Primer Premier 5.0 to amplify the full ORFs of *tadef1* and *tadef2*, based on the expression results obtained from Section 2.5. Each PCR reaction (25 μL total volume) contained: 12.5 μL 2 × Hieff Canace Gold PCR Master Mix, 1.25 μL each of forward and reverse primers (10 μM), 1 μL cDNA, and 9 μL ddH_2O. The thermocycling conditions were as follows: initial denaturation at 95 °C for 3 min; 35 cycles of denaturation at 98 °C for 10 s, annealing at 43 °C for 15 s, extension at 72 °C for 30 s; and a final extension at 72 °C for 5 min.

PCR products were separated on 1 % agarose gels, excised from the gel, and purified. The purified DNA fragments was ligated into the pTOPO-TA/Blunt vector (Aidlab Biotechnologies, Beijing) using a mixture containing 5 μL of DNA, 2 μL of vector, and 1 μL of 10 × enhancer. The ligation mixture was incubated at 37 °C for 5 min. Subsequently, 5 μL of the ligations product was transformed into *E. coli* DH5α competent cells (Yeasen Biotechnology). Cells were incubated on ice for 5 min, heat-shocked at 42 °C for 60 s, and then recovered in LB broth for 30 min at 37 °C with shaking at 200 rpm. Aliquots (100–200 μL) were spread onto LB agar plants supplemented with 100 μg/mL ampicillin and incubated overnight at 37 °C for 12 h. Single colonies were picked for sequencing, and the obtained sequences were compared with the predicted sequences.

Tadef1 and *tadef2* mature coding sequences (without signal peptides) were cloned into *pET-28b(+)* via NdeI/EcoRI sites and expressed in *E. coli* BL21(DE3). Protein expression was induced with 1 mM IPTG at 16 °C for 16 h or 37 °C for 4 h. Cells were collected, sonicated, and fractionated, and SDS–PAGE confirmed that both peptides accumulated in inclusion bodies. Pellets were washed and solubilized in PBS containing 8 M urea, and the denatured proteins were purified by Ni-NTA affinity chromatography. To obtain properly folded defensins, purified proteins (0.3–0.5 mg/mL) were refolded using a complete denaturation–renaturation protocol involving stepwise dialysis in PBS (pH 7.5) supplemented with 4 mM GSH and 0.4 mM GSSG, with sequential reductions in urea concentration (4 M → 2 M → 1 M → 0.1 M → 0 M), each step lasting 12 h at 4 °C. After final dialysis in urea-free PBS, samples were centrifuged, sterile-filtered (0.22 μm), aliquoted, and stored at –80 °C. To eliminate endotoxin contamination prior to antimicrobial assays, refolded proteins were processed using a Triton X-114 phase-separation method. Briefly, 1 % Triton X-114 was added to the protein solution and incubated with gentle rotation at 4 °C for 30 min. The mixture was then incubated at 37 °C for 10 min to induce phase separation, followed by centrifugation at 9000×g for 10 min at 25 °C. The upper aqueous phase containing endotoxin-depleted protein was carefully collected, sterile-filtered (0.22 μm), quantified, and verified via

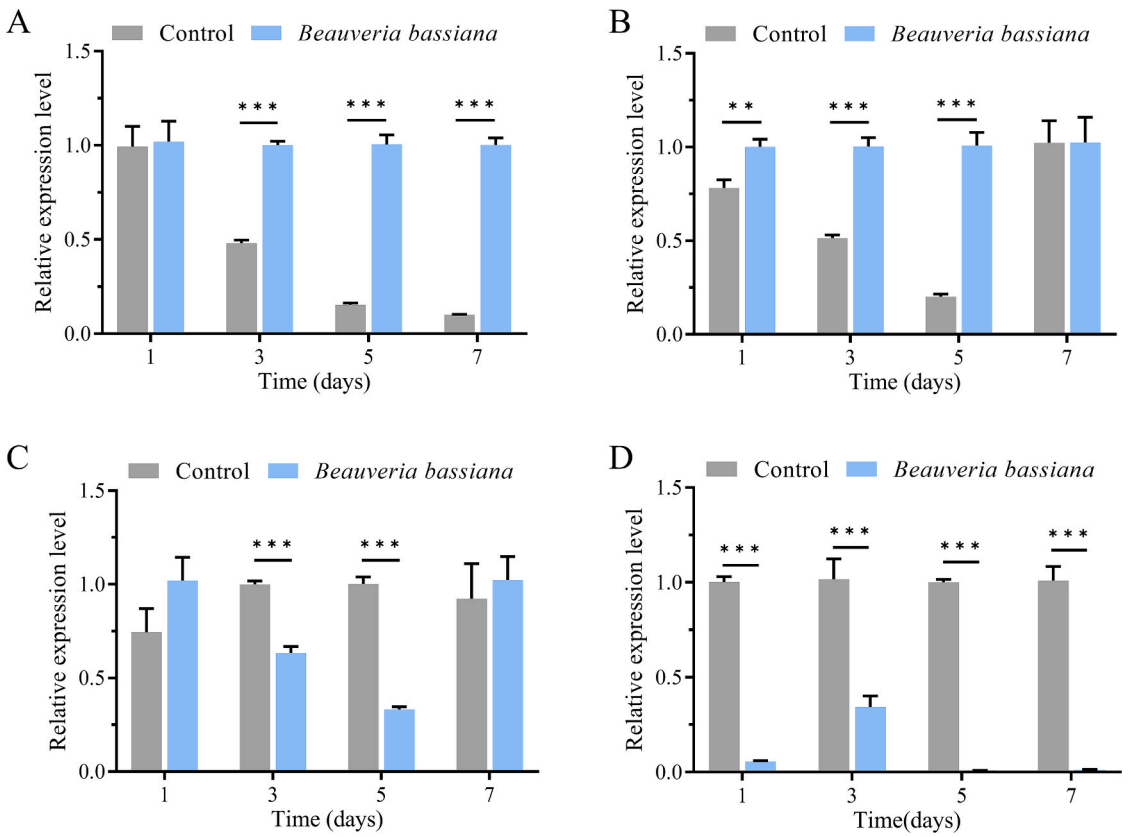


Fig. 3. Expression levels of *tadef1* (A), *tadef2* (B), *tadef3* (C), and *tadef4* (D) in second-instar larvae following *B. bassiana* infection. Data represent mean ± SEM from four biological replicates.

SDS-PAGE. Refolded and endotoxin-reduced proteins (6 mg/mL) were used for antimicrobial assays.

2.7. Antimicrobial activity of tadef1 and tadef2

Antibacterial activity was assessed using the agar well-diffusion method against *B. bassiana* and *B. thuringiensis*. PDA or LB medium (100 mL per batch) was autoclaved at 121 °C for 20 min and poured into 7 cm Petri dishes (10 mL per plate). After solidification, 200 μL of pathogen suspension (1×10^7 spores/mL or 1×10^7 CFU/mL) was evenly spread over the surface. Sterile 6 mm diameter wells were created using a metal borer. Recombinant proteins were applied at concentrations of 0.375, 0.75, 1.5, 3, and 6 mg/mL, with 10 μL per well. PBS served as a negative control. Dodine and nystatin were dissolved in DMSO and prepared at concentrations of 0, 3.125, 6.25, 12.5, 25, and 50 mg/mL to serve as positive controls for *B. thuringiensis* and *B. bassiana*, respectively. Plates were incubated at 26 °C for 48 h, and inhibition zones were recorded.

2.8. Statistical analysis

RT-qPCR expression data were log-transformed to satisfy the normality assumptions required for parametric analysis. Differences in *tadef* expression levels after *B. thuringiensis* and *B. bassiana* treatments were analyzed using independent samples *t*-tests, while variations across developmental stages and tissue types were examined using one-way ANOVA followed by Tukey's honest significant difference (HSD) test. All analyses were performed with IBM SPSS Statistics v23.0, and results are presented as mean ± standard error of the mean (SEM). Differences were considered statistically significant at $P < 0.05$ and highly significant at $P < 0.01$.

3. Results

3.1. Sequence analysis of defensin genes in T. absoluta

Through comprehensive annotation of *T. absoluta* genomic resources, four defensin-encoding genes — *tadef1* to *tadef4*— were identified (Table S3). These genes encode predicted open reading frames (ORFs) ranging from 228 to 372bp, corresponding to polypeptides of 76–124 amino acids in length. The deduced peptides are anionic, with predicted molecular weights between 8.20 and 13.4 kDa and theoretical isoelectric points (pI) ranging from 4.45 to 6.38 (Table S3). Multiple sequence alignment revealed significant homology between *tadef1–tade4* and defensins from *Spodoptera exigua* (Fig. 1A). Structural modeling of the mature peptides predicted conserved tertiary structures comprised of one α-helices and three antiparallel β-sheets, stabilized by three intramolecular disulfide bonds. This architecture aligns with the canonical insect defensin motif: C-X_{5-16}-C-X_3-C-X_{9-10}-C-X_{4-7}-CX_1-C. Chromosomal localization analysis indicated that *tadef1* and *tadef2* are located on chromosome 18, while *tadef3* and *tadef4* are on chromosome 2 (Fig. 1B). All four genes exhibit a conserved bi-exonic structure, consisting of two exons separated by a single intervening intron.

3.2. Phylogenetic analysis of T. absoluta defensins

Phylogenetic reconstruction based on representative defensin sequences from insects, arachnids, and crustaceans revealed that all four tadefs cluster within the Insecta clade, confirming their insect origin and evolutionary conservation (Fig. 2).

3.3. Pathogen-induced expression of tadef genes

RT-qPCR analysis revealed dynamic expression profiles of *tadefs* in second-instar *T. absoluta* larvae following infection with *B. bassiana* and

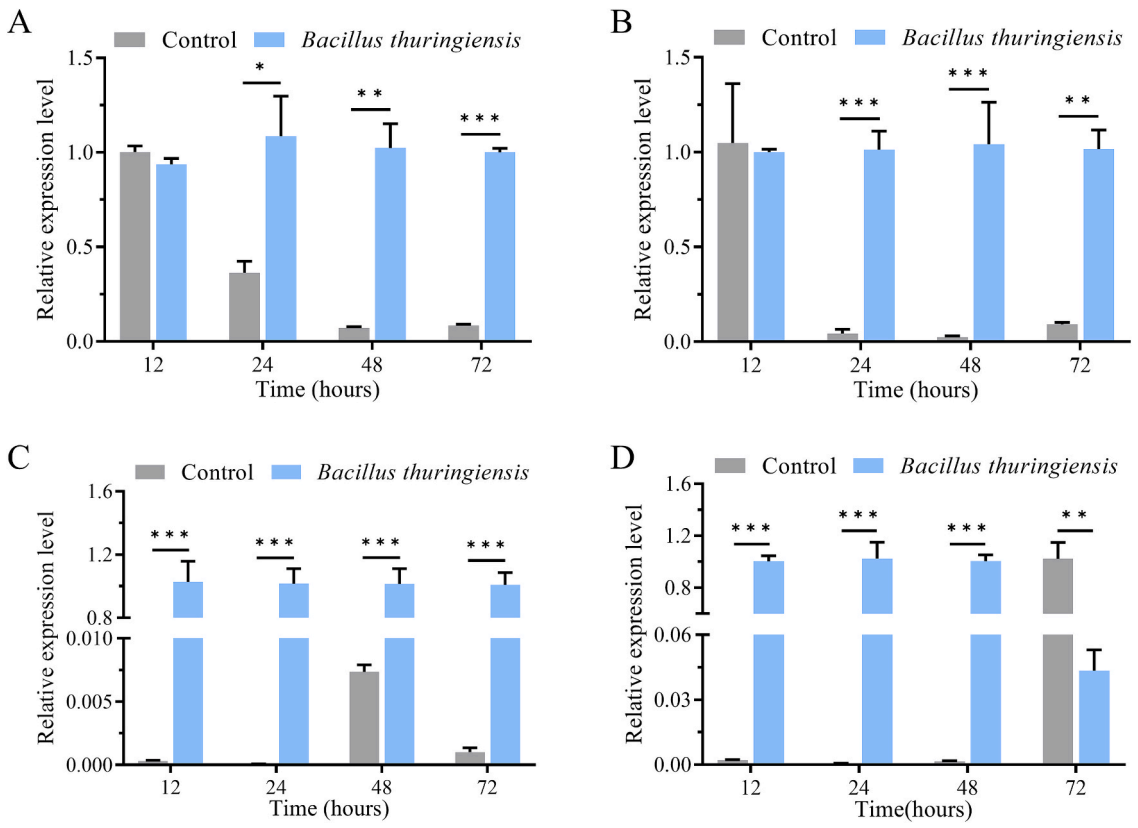


Fig. 4. Expression levels of *tadef1* (A), *tadef2* (B), *tadef3* (C), and *tadef4* (D) in second-instar larvae following *B. thuringiensis* infection. Data represent mean ± SEM from four biological replicates.

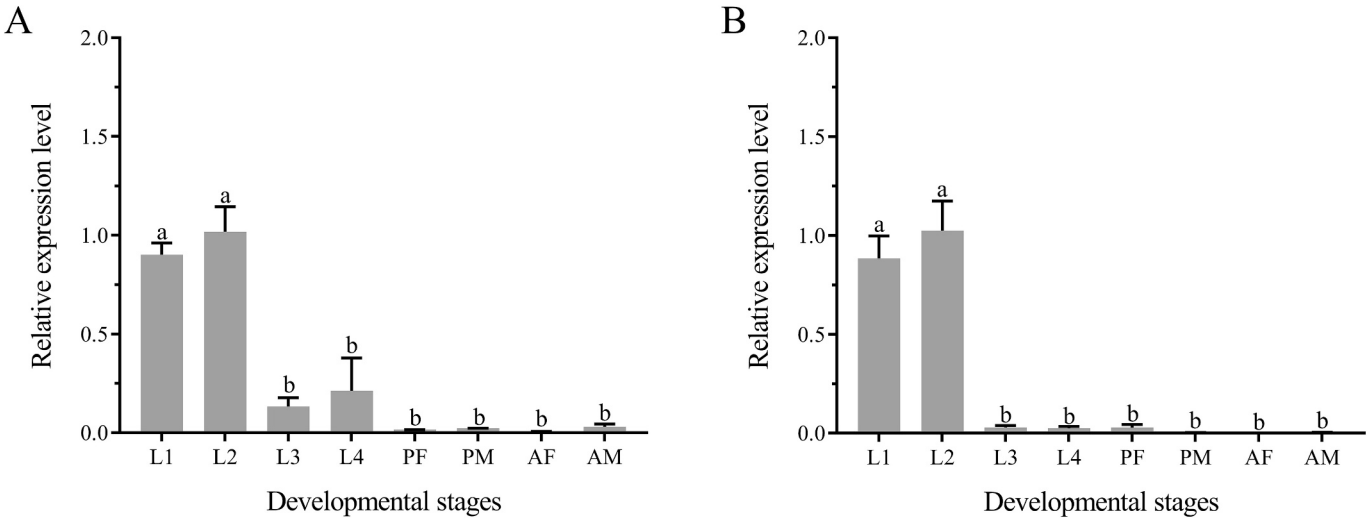


Fig. 5. Developmental and sex-specific expression of *tadef1* (A) and *tadef2* (B) across *T. absoluta* life stages: L1 (first instar), L2 (second instar), L3 (third instar), L4 (fourth instar), PF (female pupa), PM (male pupa), AF (adult female), and AM (adult male). Data represent mean ± SEM from four biological replicates.

B. thuringiensis. Upon *B. bassiana* infection, *tadef1* was significantly upregulated at 3, 5, and 7 dpi (Fig. 3A), while *tadef2* was upregulated at 1, 3, and 5 dpi (Fig. 3B). In contrast, *tadef3* showed significant downregulation at 3 and 5 dpi (Fig. 3C), and *tadef4* was suppressed at all four time points (1, 3, 5, and 7 dpi) (Fig. 3D).

Following *B. thuringiensis* infection, *tadef1* and *tadef2* were significantly induced at 24, 48, and 72 h post-treatment (Fig. 4A and Fig. 4B). *tadef3* expression was upregulated at all measured time points (12, 24, 48, and 72 h) (Fig. 4C), while *tadef4* was upregulated at 12, 24, and 48 h but significantly downregulated at 72 h (Fig. 4D).

3.4. Developmental and tissue-specific expression of tadef1 and tadef2

We further assessed the developmental, sex-specific, and tissue-specific expression profiles of *tadef1* and *tadef2* by RT-qPCR. Both genes showed peak expression during the early larval stages, particularly in first and second instars (Fig. 5). Tissue-specific analysis revealed that expression was predominantly localized to the midgut (MG), with lower expression in the epidermis (EP), fat body (FB), and malpighian tubules (MT) (Fig. 6).

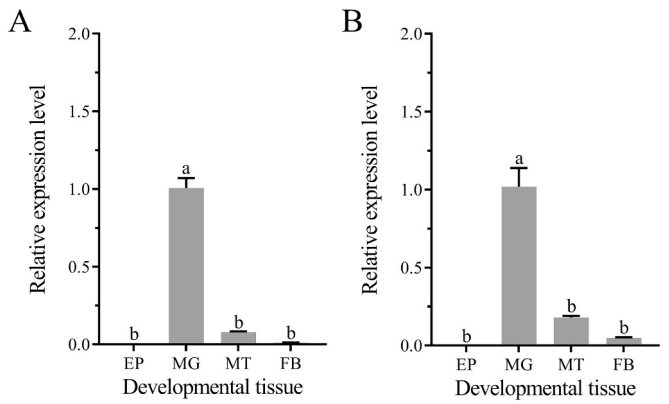


Fig. 6. Tissue-specific expression of *tadef1* (A) and *tadef2* (B) in second-instar larvae. Tissues include epidermis (EP), midgut (MG), malpighian tubule (MT), and fat body (FB). Data represent mean ± SEM from four biological replicates.

3.5. Antibacterial activity of recombinant tadef1 and tadef2

Recombinant tadef1 and tadef2 peptides were successfully expressed (Fig. 7A). Antibacterial assays revealed that both defensins exhibited dose-dependent inhibitory activity against *B. thuringiensis* (Fig. 7B, panels a and b). No inhibitory effect was observed against *B. bassiana* (Fig. 7B, panels c and d), indicating pathogen-specific activity likely confined to Gram-positive bacteria. The positive controls showed that dodine and nystatin were able to inhibit *B. thuringiensis* and *B. bassiana*, respectively (Fig. S2).

4. Discussion

In this study, we identified four defensin genes in *T. absoluta*—*tadef1*, *tadef2*, *tadef3*, and *tadef4*—each encoding peptides containing six conserved cysteine residues that conform to the canonical insect defensin motif: C-X_{5-16}-C-X_3-C-X_{9-10}-C-X_{4-7}-CX_1-C. This motif forms three intramolecular disulfide bridges (C_1–C_4, C_2–C_5, C_3–C_6) (Mulla and Tamhane, 2023; Kordaczuk et al., 2023; Bulet and Stöcklin, 2005). Genomic analysis revealed that all four genes exhibit a conserved bi-exonic structure, consisting of two exons separated by a single intron, similar to defensins in *Aedes aegypti* and *Anopheles gambiae* (Cho et al., 1997; Eggleston et al., 2000). In contrast, defensin genes in *Stomoxys calcitrans* and *Drosophila melanogaster* are intronless (Dimarcq et al., 1994; Munks et al., 2001), while defensins from *Ornithodoros moubata* defensins contain four exons and three introns (Nakajima et al., 2002).

Insect innate immune responses are rapidly activated upon pathogen recognition, yet their amplitude and profile vary with pathogen type, dose, and conditions (Bouma et al., 2010; Paixão et al., 2024; Xu et al., 2016). In *T. absoluta*, defensins displayed distinct expression patterns: *tadef1* and *tadef2* were upregulated by both *B. thuringiensis* and *B. bassiana*, whereas *tadef3* and *tadef4* were induced only by the bacterium and downregulated by the fungus, indicating pathogen-specific regulation. Consistent with this, synthetic tadef1 and tadef2 showed bactericidal but not antifungal activity, suggesting non-direct antifungal roles during fungal infection. Insect AMPs often act synergistically (Marxer et al., 2016), so co-expression of *tadef1/2* with *tadef3/4* may enhance antibacterial defense, though their interaction requires further study. These patterns align with reports in other insects: defensin upregulation by *B. thuringiensis* occurs in *T. castaneum* (Contreras et al., 2015), *S. exigua* (Zhao et al., 2023a,b), and *S. litura* (Zhong et al., 2025), and induction by *B. bassiana* is observed in *B. tabaci* (Wang et al., 2013) and *D. melanogaster* (Lemaitre et al., 1997). In this context, the specific

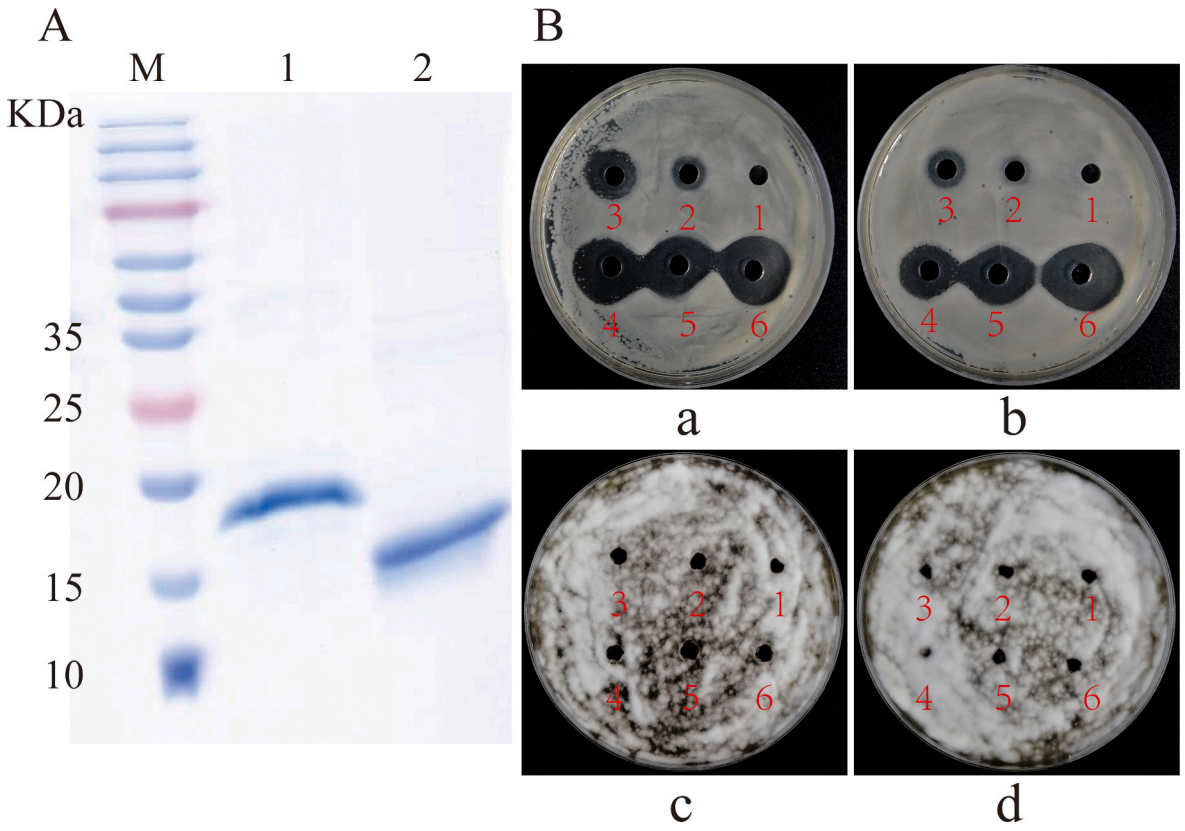


Fig. 7. Antimicrobial activity of recombinant tadef1 and tadef2. (A) Tricine-SDS-PAGE analysis of purified peptides. Lane M: molecular weight marker; Lane 1: tadef1; Lane 2: tadef2. (B) Inhibition of *B. thuringiensis* (a and b) and *B. bassiana* (c and d) by increasing concentrations of defensins: 1 (0 mg/mL), 2 (0.375 mg/mL), 3 (0.75 mg/mL), 4 (1.5 mg/mL), 5 (3.0 mg/mL), 6 (6.0 mg/mL).

downregulation of *tadef3* and *tadef4* in *T. absoluta* by *B. bassiana* is particularly striking. This may represent a pathogen-induced immunosuppression, analogous to the effect of the *Metarhizium robertsii* effector Tge1 in *Drosophila* (Lu et al., 2024), suggesting that *B. bassiana* may employ similar strategies to evade or suppress specific components of the host's immune arsenal.

The developmental regulation of AMP expression is a well-documented phenomenon across insects, often influenced by endocrine and physiological cues (Chang et al., 2021; Gong et al., 2022; Gordya et al., 2016; Jang et al., 2020; Johnston et al., 2019; Tian et al., 2010). In *T. absoluta*, the *tadef1* and *tadef2* exhibited their highest expression levels during the early larval stages, a pattern consistent with stage-specific immune gene activation observed in *A. gambiae*, *S. frugiperda* and *B. mori* (Meredith et al., 2008; Nesa et al., 2019; Tian et al., 2010; Wang et al., 2025). Such temporal peaks are thought to coincide with molting or periods of intense tissue remodeling, when immune reinforcement is transiently required. These stage-specific immune fluctuations are likely coordinated by endocrine signals, Hormonal pathways involving juvenile hormone (JH) and 20-hydroxyecdysone (20E) have been shown to coordinate these responses by modulating AMP expression via Methoprene-tolerant and related transcriptional cascades (Chang et al., 2021; Nunes et al., 2021; Tian et al., 2010). Similar stage-dependent immune activation has also been reported in *Calliphora vicina* and *D. melanogaster*, where AMP production varies markedly between larval and pupal stages (Chernysh et al., 2013; Crowley and Houck, 2002). Together, these findings suggest that the elevated defensin expression in early *T. absoluta* larvae may reflect a hormonally regulated, developmentally synchronized immune strategy to mitigate microbial challenges during vulnerable growth phases.

Tissue-specific profiling revealed that *tadef1* and *tadef2* are predominantly expressed in the midgut, positioning them at a critical immunological frontier for defense against orally acquired pathogens. This localized expression is highly relevant against *B. thuringiensis*, whose Cry toxins act directly on the midgut epithelium (Pigott and Ellar, 2007; Li et al., 2018). The induction of *tadef1* and *tadef2* by *B. thuringiensis* and the demonstrated bactericidal activity of the peptides suggest a direct role in mitigating bacterial invasion. Their defense function is likely enhanced within a coordinated immune network, for instance, their sustained expression may provide lasting antimicrobial activity after the initial reactive oxygen species (ROS) burst subsides (Wang et al., 2020; Zeng et al., 2022; Tleiss et al., 2024), and their efficacy may be modulated by digestive enzymes (Crava et al., 2015; Pinos et al., 2021). A more complex scenario is indicated by the induction of *tadef1* and *tadef2* following infection with the fungus *B. bassiana*, which primarily infects insects by penetrating the cuticle and proliferating in the hemocoel (Ortiz-Urquiza and Keyhani, 2013; Wang et al., 2021), although recent studies have also demonstrated its ability to infect via the gut (Lai et al., 2024). Despite the peptides' lack of direct antifungal activity *in vitro*, this suggests that their role during fungal challenge may not be direct hyphal inhibition but a different protective function, such as immunomodulation or preservation of epithelial integrity against virulence factors or dysbiosis.

Unlike the commonly reported cationic defensins, both tadef1 and tadef2 are predicted to encode anionic peptides, with isoelectric points of 4.66 and 4.54, respectively. Cationic AMP frequently exhibit antibacterial activity against Gram-positive bacteria, such as those characterized in *P. xylostella* and *Amblyomma hebraeum*, primarily through binding to negatively charged bacterial membranes and forming disruptive pores (Koehbach, 2017; Seufi et al., 2011). In contrast, the mode of action of anionic AMP remains poorly understood. Current hypotheses propose that anionic antimicrobial peptides require complexation with metal ions (e.g., Zn^{2+}) to partially neutralize or even reverse their net negative charge, thereby enabling effective interaction with bacterial membranes and exerting antibacterial activity (Brogden et al., 2003, 1997, 1996; Harris et al., 2009). Such a mechanism could explain why tadef1/2 retain activity against Gram-positive bacteria despite their anionic nature (Fig. 7B). However, their lack of activity against fungi may be attributed to the highly complex and multilayered fungal cell wall, which restricts defensin access to the inner membrane and thus confers inherent resistance to many peptide-based antimicrobial mechanisms (Harris et al., 2009).

In *T. absoluta*, *tadef1* and *tadef2* were strongly induced by *B. thuringiensis* and *B. bassiana*, with the highest expression in 1st–2nd instar larvae and midgut tissue. Their inducibility and strategic midgut localization indicate that they may serve as promising molecular targets for enhancing microbial control, for instance through RNAi-mediated defensin suppression or the co-application of entomopathogens with immune-modulatory agents. However, the present study is limited to laboratory assays, and we did not conduct greenhouse or field experiments to determine whether manipulating defensin expression can improve the performance of entomopathogens under realistic environmental conditions. Because field efficacy of microbial agents is strongly influenced by host immunity, environmental variability, and pathogen persistence, the absence of such trials restricts our ability to directly translate the molecular findings into practical biocontrol recommendations. Future studies incorporating RNAi knockdown together with controlled greenhouse tests and subsequent field validation will be essential to assess whether defensin-targeted immune modulation can reliably enhance entomopathogen-based suppression of *T. absoluta*. Despite these limitations, our results provide a mechanistic basis for designing such applied studies and support the potential value of defensin-focused strategies in integrated pest management.

5. Conclusions

In summary, our findings provide novel molecular insights into the immune repertoire of *T. absoluta*, with particular emphasis on defensin-mediated responses to microbial infection. The distinct expression patterns and functional divergence observed among tadef paralogs indicate evolutionary adaptation to diverse microbial threats. These results not only advance our understanding of Lepidopteran immunity mechanisms but also hold potential for informing the development of pathogen-based biocontrol strategies against this globally invasive pest.

CRediT authorship contribution statement

Bo Xu: Writing – original draft, Visualization, Software, Methodology, Investigation, Formal analysis, Data curation. **Guohui Zhang:** Conceptualization. **Nannan Zhu:** Methodology, Investigation. **Jörg Romeis:** Writing – review & editing, Supervision. **Jana Collatz:** Writing – review & editing, Supervision. **Guifen Zhang:** Supervision, Resources, Funding acquisition. **Cong Huang:** Writing – review & editing, Validation, Supervision, Resources, Project administration, Conceptualization. **Yibo Zhang:** Writing – review & editing, Supervision, Resources, Project administration, Funding acquisition. **Fanghao Wan:** Resources.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

This work was supported by the National Key Research and Development Project of China (grant numbers 2022YFC2601000, and 2021YFD1400200), Central Public-interest Scientific Institution Basal Research Fund (No. S2025XM32) and the Project of the Engineering Research Center of Ecology and Agricultural Use of Wetland, Ministry of Education (grant numbers KFG202421).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.biocontrol.2025.105944.

References

Ali Mohammadie Kojour, M., Han, Y.S., Jo, Y.H., 2020. An overview of insect innate immunity. Entomol. Res. 50, 282–291. https://doi.org/10.1111/1748-5967.12437.

Biondi, A., Guedes, R.N.C., Wan, F.H., Desneux, N., 2018. Ecology, worldwide spread, and management of the invasive south American tomato pinworm, *Tuta absoluta*: past, present, and future. Annu. Rev. Entomol. 63, 239–258. https://doi.org/10.1146/annurev-ento-031616-034933.

Blum, M., Andreeva, A., Florentino, L.C., Chuguransky, S.R., Grego, T., Hobbs, E., Pinto, B.L., Orr, A., Paysan-Lafosse, T., Ponamareva, I., Salazar, G.A., Bordin, N., Bork, P., Bridge, A., Colwell, L., Gough, J., Haft, D.H., Letunic, I., Llinares-López, F., Marchler-Bauer, A., Meng-Papaxanthos, L., Mi, H., Natale, D.A., Orengo, C.A., Pandurangan, A.P., Piovesan, D., Rivoire, C., Sigrist, C.J.A., Thanki, N., Thibaud-Nissen, F., Thomas, P.D., Tosatto, S.C.E., Wu, C.H., Bateman, A., 2025. InterPro: The protein sequence classification resource in 2025. Nucleic Acids Res. 53, D444–D456. https://doi.org/10.1093/nar/gkae1082.

Bouma, H.R., Carey, H.V., Kroese, F.G.M., 2010. Hibernation: The immune system at rest? J. Leukoc. Biol. 88, 619–624. https://doi.org/10.1189/jlb.0310174.

Brogden, K.A., Ackermann, M., Huttner, K.M., 1997. Small, anionic, and charge-neutralizing propeptide fragments of zymogens are antimicrobial. Antimicrob. Agents Chemother. 41, 1615–1617. https://doi.org/10.1128/AAC.41.7.1615.

Brogden, K.A., Ackermann, M., McCray, P.B., Tack, B.F., 2003. Antimicrobial peptides in animals and their role in host defences. Int. J. Antimicrob. Agents 22, 465–478. https://doi.org/10.1016/s0924-8579(03)00180-8.

Brogden, K.A., De Lucca, A.J., Bland, J., Elliott, S., 1996. Isolation of an ovine pulmonary surfactant-associated anionic peptide bactericidal for *Pasteurella haemolytica*. PNAS 93, 412–416. https://doi.org/10.1073/pnas.93.1.412.

Bulet, P., Hetru, C., Dimarcq, J.L., Hoffmann, D., 1999. Antimicrobial peptides in insects; structure and function. Dev. Comp. Immunol. 23, 329–344. https://doi.org/10.1016/S0145-305X(99)00015-4.

Bulet, P., Stöcklin, R., 2005. Insect antimicrobial peptides: Structures, properties and gene regulation. Protein Pept. Lett. 12, 3–11. https://doi.org/10.2174/0929866053406011.

Campos, M.R., Rodrigues, A.R.S., Silva, W.M., Silva, T.B.M., Silva, V.R.F., Guedes, R.N.C., Siqueira, H.A.A., 2014. Spinosad and the tomato borer *Tuta absoluta*: a bioinsecticide, an invasive pest threat, and high insecticide resistance. PLoS One 9, e103235. https://doi.org/10.1371/journal.pone.0103235.

Chang, M.M., Wang, Y.H., Yang, Q.T., Wang, X.L., Wang, M., Raikhel, A.S., Zou, Z., 2021. Regulation of antimicrobial peptides by juvenile hormone and its receptor, Methoprene-tolerant, in the mosquito *Aedes aegypti*. Insect Biochem. Mol. Biol. 128, 103509. https://doi.org/10.1016/j.ibmb.2020.103509.

Chen, C., Wu, Y., Li, J., Wang, X., Zeng, Z., Xu, J., Liu, Y., Feng, J., Chen, H., He, Y., Xia, R., 2023. TBtools-II: A "one for all, all for one" bioinformatics platform for biological big-data mining. Mol. Plant 16, 1733–1742. https://doi.org/10.1016/j.molp.2023.09.010.

Chernysh, S.I., Simonenko, N.P., Braun, A., Meister, M., 2013. Developmental variability of the antibacterial response in larvae and pupae of *Calliphora vicina* (Diptera: Calliphoridae) and *Drosophila melanogaster* (Diptera: Drosophilidae). Eur. J. Entomol. 92, 203–209.

Cho, W.L., Fu, T.F., Chiou, J.Y., Chen, C., 1997. Molecular characterization of a defensin gene from the mosquito, *Aedes Aegypti*. Insect Biochem. Mol. Biol. 27, 351–358. https://doi.org/10.1016/S0965-1748(97)00017-9.

Contreras, E., Benito-Jardón, M., López-Galiano, M.J., Real, M.D., Rausell, C., 2015. *Tribolium castaneum* immune defense genes are differentially expressed in response to *Bacillus thuringiensis* toxins sharing common receptor molecules and exhibiting disparate toxicity. Dev. Comp. Immunol. 50, 139–145. https://doi.org/10.1016/j.dci.2015.02.005.

Crava, C.M., Jakubowska, A.K., Escriche, B., Herrero, S., Bel, Y., 2015. Dissimilar regulation of antimicrobial proteins in the midgut of *Spodoptera exigua* larvae challenged with *Bacillus thuringiensis* toxins or baculovirus. PLoS One 10, e0125991. https://doi.org/10.1371/journal.pone.0125991.

Crowley, L.D., Houck, M.A., 2002. The immune response of larvae and pupae of *Calliphora vicina* (Diptera: Calliphoridae), upon administered insult with *Escherichia coli*. J. Med. Entomol. 39, 931–934. https://doi.org/10.1603/0022-2585-39.6.931.

Cytryńska, M., Wojda, I., Jakubowicz, T., 2016. Chapter 9 - how insects combat infections. In: Ballarin, L., Cammarata, M. (Eds.), Lessons in Immunity. Academic Press, New York, pp. 117–128. https://doi.org/10.1016/B978-0-12-803252-7.00009-6.

Desneux, N., Wajnberg, E., Wyckhuys, K.A.G., Burgio, G., Arpaia, S., Narváez-Vasquez, C. A., González-Cabrera, J., Catalán Ruescas, D., Tabone, E., Frandon, J., Pizzol, J., Poncet, C., Cabello, T., Urbaneja, A., 2010. Biological invasion of European tomato crops by *Tuta absoluta*: Ecology, geographic expansion and prospects for biological control. J. Pest. Sci. 83, 197–215. https://doi.org/10.1007/s10340-010-0321-6.

Díaz-Garrido, P., Cárdenas-Guerra, R.E., Martínez, I., Poggio, S., Rodríguez-Hernández, K., Rivera-Santiago, L., Ortega-López, J., Sánchez-Esquivel, S., Espinoza, B., 2021. Differential activity on trypanosomatid parasites of a novel recombinant defensin type 1 from the insect *Triatoma (Meccus) pallidipennis*. Insect Biochem. Mol. Biol. 139, 103673. https://doi.org/10.1016/j.ibmb.2021.103673.

Dimarcq, J.L., Hoffmann, D., Meister, M., Bulet, P., Lanot, R., Reichhart, J.M., Hoffmann, J.A., 1994. Characterization and transcriptional profiles of a *Drosophila* gene encoding an insect defensin. A study in insect immunity. Eur. J. Biochem. 221, 201–209. https://doi.org/10.1111/j.1432-1033.1994.tb18730.x.

Eggleston, P., Lu, W., Zhao, Y., 2000. Genomic organization and immune regulation of the defensin gene from the mosquito, *Anopheles Gambiae*. Insect Mol. Biol. 9, 481–490. https://doi.org/10.1046/j.1365-2583.2000.00212.x.

Ganz, T., Lehrer, R.I., 1994. Defensins. Curr. Opin. Immunol. 6, 584–589. https://doi.org/10.1016/0952-7915(94)90145-7.

Gong, T., Du, J., Li, S.-W., Huang, H., Qi, X.L., 2022. Identification and functional analysis of a defensin *CcDef2* from *Coridius chinensis*. Int. J. Mol. Sci. 23, 2789. https://doi.org/10.3390/ijms23052789.

Gordya, N.A., Nesin, A.P., Simonenko, N.P., Chernysh, S.I., 2016. Regulation of antimicrobial peptide synthesis in larvae of *Calliphora vicina* (Diptera, Calliphoridae): a dose-dependent effect of ecdysteroids. J. Evol. Biochem. Physiol. 52, 292–298. https://doi.org/10.1134/S0022093016040037.

Guedes, R.N.C., Picanço, M.C., 2012. The tomato borer *Tuta absoluta* in South America: Pest status, management and insecticide resistance. EPPO Bull. 42, 211–216. https://doi.org/10.1111/epp.2557.

Guedes, R.N.C., Roditakis, E., Campos, M.R., Haddi, K., Bielza, P., Siqueira, H.A.A., Tsagkarakou, A., Vontas, J., Nauen, R., 2019. Insecticide resistance in the tomato pinworm *Tuta absoluta*: patterns, spread, mechanisms, management and outlook. J. Pest. Sci. 92, 1329–1342. https://doi.org/10.1007/s10340-019-01086-9.

Hanson, M.A., Lemaitre, B., Unckless, R.L., 2019. Dynamic evolution of antimicrobial peptides underscores trade-offs between immunity and ecological fitness. Front. Immunol. 10, 2620. https://doi.org/10.3389/fimmu.2019.02620.

Harris, F., Dennison, S.R., Phoenix, D.A., 2009. Anionic antimicrobial peptides from eukaryotic organisms. Curr. Protein Pept. Sci. 10, 585–606. https://doi.org/10.2174/138920309789630589.

He, Y., Cao, X., Li, K., Hu, Y., Chen, Y., Blissard, G., Kanost, M.R., Jiang, H., 2015. A genome-wide analysis of antimicrobial effector genes and their transcription patterns in *Manduca sexta*. Insect Biochem. Mol. Biol. 62, 23–37. https://doi.org/10.1016/j.ibmb.2015.01.015.

Hoffmann, J.A., 1995. Innate immunity of insects. Curr. Opin. Immunol. 7, 4–10. https://doi.org/10.1016/0952-7915(95)80022-0.

Jang, H.A., Park, K.B., Kim, B.B., Ali Mohammadie Kojour, M., Bae, Y.M., Baliarsingh, S., Lee, Y.S., Han, Y.S., Jo, Y.H., 2020. In silico identification and expression analyses of Defensin genes in the mealworm beetle. Entomol. Res. 50, 575–585. https://doi.org/10.1111/1748-5967.12468.

Jo, Y.H., Lee, J.H., Patnaik, B.B., Keshavarz, M., Lee, Y.S., Han, Y.S., 2021. Autophagy in *Tenebrio molitor* Immunity: Conserved antimicrobial functions in insect defenses. Front. Immunol. 12, 667664. https://doi.org/10.3389/fimmu.2021.667664.

Johnston, P.R., Paris, V., Rolff, J., 2019. Immune gene regulation in the gut during metamorphosis in a holo- versus a hemimetabolous insect. Philos. Trans. R. Soc. Lond. B Biol. Sci. 374, 1783. https://doi.org/10.1098/rstb.2019.0073.

Kaneko, Y., Tanaka, H., Ishibashi, J., Iwasaki, T., Yamakawa, M., 2008. Gene expression of a novel defensin antimicrobial peptide in the silkworm, *Bombyx mori*. Biosci. Biotech. Bioch. 72, 2353–2361. https://doi.org/10.1271/bbb.80263.

Koehbach, J., 2017. Structure-activity relationships of insect defensins. Front. Chem. 5, 45. https://doi.org/10.3389/fchem.2017.00045.

Kordaczuk, J., Sułek, M., Mak, P., Śmiałek-Bartyzel, J., Hułas-Stasiak, M., Wojda, I., 2023. Defence response of *Galleria mellonella* larvae to oral and intrahemocelic infection with *Pseudomonas entomophila*. Dev. Comp. Immunol. 147, 104749. https://doi.org/10.1016/j.dci.2023.104749.

Lai, Y., Zheng, W., Zheng, Y., Lu, H., Qu, S., Wang, L., Li, M., Wang, S., 2024. Unveiling a novel entry gate: Insect foregut as an alternative infection route for fungal entomopathogens. Innovation 5, 100644. https://doi.org/10.1016/j.xinn.2024.100644.

Lambert, J., Keppi, E., Dimarcq, J.L., Wicker, C., Reichhart, J.M., Dunbar, B., Lepage, P., Van Dorsselaer, A., Hoffmann, J., Fothergill, J., 1989. Insect immunity: Isolation from immune blood of the dipteran *Phormia terranovae* of two insect antibacterial peptides with sequence homology to rabbit lung macrophage bactericidal peptides. PNAS 86, 262–266. https://doi.org/10.1073/pnas.86.1.262.

Lamberty, M., Ades, S., Uttenweiler-Joseph, S., Brookhart, G., Bushey, D., Hoffmann, J. A., Bulet, P., 1999. Insect immunity. Isolation from the lepidopteran *Heliothis virescens* of a novel insect defensin with potent antifungal activity. J. Biol. Chem. 274, 9320–9326. https://doi.org/10.1074/jbc.274.14.9320.

Lemaitre, B., 2004. The road to toll. Nat. Rev. Immunol. 4, 521–527. https://doi.org/10.1038/nri1390.

Lemaitre, B., Reichhart, J.M., Hoffmann, J.A., 1997. *Drosophila* host defense: Differential induction of antimicrobial peptide genes after infection by various classes of microorganisms. PNAS 94, 14614–14619. https://doi.org/10.1073/pnas.94.26.14614.

Leulier, F., Lemaitre, B., 2008. Toll-like receptors — taking an evolutionary approach. Nat. Rev. Genet. 9, 165–178. https://doi.org/10.1038/nrg2303.

Li, S., Xu, X., Shakeel, M., Xu, J., Zheng, Z., Zheng, J., Yu, X., Zhao, Q., Jin, F., 2018. *Bacillus thuringiensis* suppresses the humoral immune system to overcome defense mechanism of *Plutella xylostella*. Front. Physiol. 9. https://doi.org/10.3389/fphys.2018.01478.

Lu, M., Wei, D., Shang, J., Li, S., Song, S., Luo, Y., Tang, G., Wang, C., 2024. Suppression of *Drosophila* antifungal immunity by a parasite effector via blocking GNBP3 and GNBP-like 3, the dual receptors for β-glucans. Cell Rep. 43, 113642. https://doi.org/10.1016/j.celrep.2023.113642.

Marxer, M., Vollenweider, V., Schmid-Hempel, P., 2016. Insect antimicrobial peptides act synergistically to inhibit a trypanosome parasite. Philos. Trans. R. Soc. Lond. B Biol. Sci. 371, 20150302. https://doi.org/10.1098/rstb.2015.0302.

Matova, N., Anderson, K.V., 2006. Rel/NF-κB double mutants reveal that cellular immunity is central to *Drosophila* host defense. PNAS 103, 16424–16429. https://doi.org/10.1073/pnas.0605721103.

Matsuyama, K., Natori, S., 1988. Molecular cloning of cDNA for sapecin and unique expression of the sapecin gene during the development of *Sarcophaga peregrina*. J. Biol. Chem. 263, 17117–17121. https://doi.org/10.1016/S0021-9258(18)37506-9.

Meredith, J.M., Hurd, H., Lehane, M.J., Eggleston, P., 2008. The malaria vector mosquito *Anopheles gambiae* expresses a suite of larval-specific defensin genes. Insect Mol. Biol. 17, 103–112. https://doi.org/10.1111/j.1365-2583.2008.00786.x.

Mulla, J.A., Tamhane, V.A., 2023. Novel insights into plant defensin ingestion induced metabolic responses in the polyphagous insect pest *Helicoverpa armigera*. Sci. Rep. 13, 3151. https://doi.org/10.1038/s41598-023-29250-3.

Munks, R.J.L., Hamilton, J.V., Lehane, S.M., Lehane, M.J., 2001. Regulation of midgut defensin production in the blood-sucking insect *Stomoxys calcitrans*. Insect Mol. Biol. 10, 561–571. https://doi.org/10.1046/j.0962-1075.2001.00296.x.

Nakajima, Y., Van Der Goes van Naters-Yasui, A., Taylor, D., Yamakawa, M., 2002. Antibacterial peptide defensin is involved in midgut immunity of the soft tick, *Ornithodoros moubata*. Appl. Entomol. Zool. 11, 611–618. https://doi.org/10.1046/j.1365-2583.2002.00372.x.

Nesa, J., Sadat, A., Buccini, D.F., Kati, A., Mandal, A.K., Franco, O.L., 2019. Antimicrobial peptides from *Bombyx mori*: A splendid immune defense response in silkworms. RSC Adv. 10, 512–523. https://doi.org/10.1039/c9ra06864c.

Nunes, C., Sucena, É., Koyama, T., 2021. Endocrine regulation of immunity in insects. FEBS J. 288, 3928–3947. https://doi.org/10.1111/febs.15581.

Ortiz-Urquiza, A., Keyhani, N.O., 2013. Action on the surface: Entomopathogenic fungi versus the Insect cuticle. Insects 4, 357–374. https://doi.org/10.3390/insects4030357.

Paixão, F.R.S., Falvo, M.L., Huarte-Bonnet, C., Santana, M., García, J.J., Fernandes, É.K. K., Pedrini, N., 2024. Pathogenicity of microsclerotia from *Metarhizium robertsii* against *Aedes aegypti* larvae and antimicrobial peptides expression by mosquitoes during fungal-host interaction. Acta Trop. 249, 107061. https://doi.org/10.1016/j.actatropica.2023.107061.

Patyra, E., Kwiatek, K., 2023. Insect meals and insect antimicrobial peptides as an alternative for antibiotics and growth promoters in livestock production. Pathogens 12, 854. https://doi.org/10.3390/pathogens12060854.

Pigott, C.R., Ellar, D.J., 2007. Role of receptors in *Bacillus thuringiensis* crystal toxin activity. Microbiol. Mol. Biol. Rev. 71, 255–281. https://doi.org/10.1128/MMBR.00034-06.

Pinos, D., Andrés-Garrido, A., Ferré, J., Hernández-Martínez, P., 2021. Response mechanisms of invertebrates to *Bacillus thuringiensis* and its pesticidal proteins. Microbiol. Mol. Biol. Rev. 85 (1), e00007–e20. https://doi.org/10.1128/mmbr.00007-20.

Seufi, A.M., Hafez, E.E., Galal, F.H., 2011. Identification, phylogenetic analysis and expression profile of an anionic insect defensin gene, with antibacterial activity, from bacterial-challenged cotton leafworm, *Spodoptera littoralis*. BMC Mol. Biol. 12, 47. https://doi.org/10.1186/1471-2199-12-47.

Silva, J.E., Assis, C.P.O., Ribeiro, L.M.S., Siqueira, H.A.A., 2016. Field-evolved resistance and cross-resistance of Brazilian *Tuta absoluta* (Lepidoptera: Gelechiidae) populations to diamide insecticides. J. Econ. Entomol. 109, 2190–2195. https://doi.org/10.1093/jee/tow161.

Tanji, T., Yun, E.Y., Ye, W., 2010. Heterodimers of NF-κB transcription factors DIF and Relish regulate antimicrobial peptide genes in *Drosophila*. PNAS 107, 14715–14720. https://doi.org/10.1073/pnas.1009473107.

Thevissen, K., Warnecke, D.C., François, I.E.J.A., Leipelt, M., Heinz, E., Ott, C., Zähringer, U., Thomma, B.P.H.J., Ferket, K.K.A., Cammue, B.P.A., 2004. Defensins from insects and plants interact with fungal glucosylceramides. J. Biol. Chem. 279, 3900–3905. https://doi.org/10.1074/jbc.M311165200.

Tian, L., Guo, E., Diao, Y., Zhou, S., Peng, Q., Cao, Y., Ling, E., Li, S., 2010. Genome-wide regulation of innate immunity by juvenile hormone and 20-hydroxyecdysone in the *Bombyx* fat body. BMC Genomics 11, 549. https://doi.org/10.1186/1471-2164-11-549.

Tleiss, F., Montanari, M., Milleville, R., Pierre, O., Royet, J., Osman, D., Gallet, A., Kurz, C.L., 2024. Spatial and temporal coordination of Duox/TrpA1/Dh31 and IMD pathways is required for the efficient elimination of pathogenic bacteria in the intestine of *Drosophila* larvae. Elife 13, RP98716. https://doi.org/10.7554/eLife.98716.

Tonk, M., Knorr, E., Cabezas-Cruz, A., Valdés, J.J., Kollewe, C., Vilcinskas, A., 2015. *Tribolium castaneum* defensins are primarily active against Gram-positive bacteria. J. Invertebr. Pathol. 132, 208–215. https://doi.org/10.1016/j.jip.2015.10.009.

Volkoff, A.N., Rocher, J., d'Alençon, E., Bouton, M., Landais, I., Quesada-Moraga, E., Vey, A., Fournier, P., Mita, K., Devauchelle, G., 2003. Characterization and transcriptional profiles of three *Spodoptera frugiperda* genes encoding cysteine-rich peptides. A new class of defensin-like genes from lepidopteran insects? Gene 319, 43–53. https://doi.org/10.1016/S0378-1119(03)00789-3.

Wang, G., Ran, J., Jia, C., Mohamed, A., Paredes-Montero, J.R., Al-Akeel, R.K., Zang, L., Zhang, W., Keyhani, N.O., Eleftherianos, I., 2025. Developmental transcriptomics reveals stage-specific immune gene expression profiles in *Spodoptera frugiperda*. Sci. Rep. 15, 25044. https://doi.org/10.1038/s41598-025-06939-1.

Wang, M.H., Ismoilov, K., Liu, W.X., Bai, M., Bai, X.S., Chen, B., Chen, H.L., Chen, H.S., Dong, Y.C., Fang, K., Gui, F.R., Huang, G.H., Jiang, C.M., Jiang, H.B., Li, X.W., Luo, C., Lu, Z.Z., Lu, Y.B., Ma, D.Y., Pu, D.Q., 2024. *Tuta absoluta* management in China: Progress and prospects. Entomol. Gen. 44, 269–278. https://doi.org/10.1127/entomologia/2024/2362.

Wang, H., Peng, H., Li, W., Cheng, P., Gong, M., 2021. The Toxins of *Beauveria bassiana* and the strategies to improve their virulence to insects. Front. Microbiol. 12. https://doi.org/10.3389/fmicb.2021.705343.

Wang, R.J., Chen, K.K., Xing, L.S., Lin, Z., Lu, Z.Q., 2020. Reactive oxygen species and antimicrobial peptides are sequentially produced in silkworm midgut in response to bacterial infection. Dev. Comp. Immunol. 110, 103720. https://doi.org/10.1016/j.dci.2020.103720.

Wang, Z.Z., Shi, M., Ye, X.Q., Chen, M.Y., Chen, X.X., 2013. Identification, characterization and expression of a defensin-like antifungal peptide from the whitefly *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae). Insect Mol. Biol. 22, 297–305. https://doi.org/10.1111/imb.12021.

Wu, Q., Patočka, J., Kuča, K., 2018. Insect antimicrobial peptides, a mini review. Toxins (basel) 10, 461. https://doi.org/10.3390/toxins10110461.

Xu, X.X., Zhang, Y.Q., Freed, S., Yu, J., Gao, Y.F., Wang, S., Ouyang, L.N., Ju, W.Y., Jin, F. L., 2016. An anionic defensin from *Plutella xylostella* with potential activity against *Bacillus thuringiensis*. Bull. Entomol. Res. 106, 790–800. https://doi.org/10.1017/S0007485316000596.

Zeng, T., Jaffar, S., Xu, Y., Qi, Y., 2022. The intestinal immune defense system in insects. Int. J. Mol. Sci. 23, 15132. https://doi.org/10.3390/ijms232315132.

Zhang, Y., Li, H., Han, P., Tian, X., Wang, H., Geng, L., Zhang, J., Liu, W., Wan, F., Guedes, R.-N., Nicolas, D., Zhang, G., 2025. Monitoring the insecticide susceptibility of a newly introduced invasive species, *Tuta absoluta* (Meyrick), in China. Crop Prot. 189, 107041. https://doi.org/10.1016/j.cropro.2024.107041.

Zhao, D., Wu, H., Li, Y., Wang, Q., Ji, Y., Guo, X., Guo, W., 2023a. Effects of the pyrE deletion mutant from *Bacillus thuringiensis* on gut microbiota and immune response of *Spodoptera exigua*. Front. Microbiol. 14. https://doi.org/10.3389/fmicb.2023.1182699.

Zhao, L., Niu, J., Feng, D., Wang, X., Zhang, R., 2023b. Immune functions of pattern recognition receptors in Lepidoptera. Front. Immunol. 14. https://doi.org/10.3389/fimmu.2023.1203061.

Zhong, J., Li, J., Wu, D., Deng, X., Lu, Y., Yu, X.Q., 2025. Characterization and functional analysis of *Spodoptera litura* galectins. Pest Manag. Sci. 81, 3148–3161. https://doi.org/10.1002/ps.8685.