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Cloning and Expression Pattern of the Carboxylesterase Gene *GmCarE4a* in *Galleria mellonella* (Lepidoptera: Pyralidae) under Chemical Stress

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ABSTRACT

To investigate the response characteristics of the carboxylesterase gene *GmCarE4a* in *Galleria mellonella* to insecticide stress, we used a previously generated transcriptome dataset of *G. mellonella* from our research group. The complete coding sequence (CDS) of the *GmCarE4a* in *G. mellonella* was cloned by polymerase chain reaction (PCR), followed by bioinformatics analysis and phylogenetic tree construction. Additionally, reverse transcription quantitative polymerase chain reaction (RT-qPCR) was used to analyse the expression patterns of this gene across different developmental stages, tissue locations, and under varying concentrations of chlorantraniliprole stress. The results showed that the CDS region of *GmCarE4a* from *G. mellonella* spans 1743 bp (CNCB: OMIX013348), encoding 581 amino acids. The CDS possesses the typical conserved domains of carboxylesterases, belongs to the *β* family, and exhibits the closest phylogenetic relationship to GmCarE4a of *Achroia grisella. GmCarE4a* exhibits differential spatial and temporal expression in *G. mellonella,* with the highest expression levels observed in late-instar larvae and epidermal tissues. Under chlorantraniliprole stress, its expression levels in the treated groups were significantly higher than in the control group. These findings suggest that *GmCarE4a* may play a key role in the detoxification and metabolism of *G. mellonella.*

Keywords: Galleria mellonella, chemical pesticide, molecular cloning, bioinformatics, expression characteristics.

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INTRODUCTION

Galleria mellonella (Linnaeus, 1758), belongs to the family Pyralidae in the order Lepidoptera and is a globally distributed pest of honey bee colonies (Kwadha, Ong'amo, Ndegwa, Raina, & Fombon, 2017). The significant economic value of *Apis cerana* (Fabricius, 1793), a key pollinator (Patel, Pauli, Biggs, Barbour, & Boruff, 2021), is severely threatened by *G. mellonella*. The larvae damage honeycomb and weakening colonies (Wojda, Staniec, Sułek, & Kordaczuk, 2020), leading to direct economic losses for beekeepers and indirectly threatening the economic value of pollination-dependent crops (Cao et al., 2024). Current control measures for *G. mellonella* primarily fall into three categories: physical, biological, and chemical control. Among these, chemical control is the most widely adopted due to its rapid effectiveness and ease of application (Su, Chen, Zhao, Hua, & Zeng, 2021). Chlorantraniliprole is a highly effective, low-toxicity insecticide that has gained increasing popularity in recent years (Wan, Li, Long, Smagghe, & Liu, 2025). It induces death by activating insect ryanodine receptors (Kong et al., 2024; Wan, Li, Long, Smagghe, & Liu, 2025), causing sustained muscle contraction (Chen, Zhang, Wang, Zhang, & Zhang, 2025). However, long-term exclusive use of chemical pesticides has led to the development of resistance in various pests. *Cnaphalocrocis medinalis* (Guenée, 1854) has developed 64.9- to 113.7-fold resistance to chlorantraniliprole (Chen et al., 2024). In 2021, field populations of *Spodoptera frugiperda* (Smith, 1797) in Guangzhou exhibited moderate resistance (Lv et al., 2021). Five field populations of *Tuta absoluta* (Meyrick, 1917) in Xinjiang, China also showed widespread resistance to chlorantraniliprole (Li et al., 2025). Therefore, elucidating the molecular mechanisms of resistance is essential to provide a scientific basis for green pest control technologies.

Carboxylesterases (CarEs) are a class of hydrolytic enzymes widely distributed in insects that participates in the metabolism of exogenous toxins, such as insecticides and plant secondary metabolites, and thus contribute to detoxication (Yan, Cui, & Qiao, 2009; Jackson et al., 2013). The active site of CarE contains a conserved serine residue that catalyzes the cleavage and formation of ester bonds (Kaur et al., 2024). CarE exhibits various functions, including participating in intracellular lactones in microorganisms and regulating lipid homeostasis, ester metabolism, growth, and neurotransmitter transmission in mammals (Johan, Rahman, Kamarudin, & Ali, 2021; Wang et al., 2018). In insects, CarEs have multiple physiological roles, participating in fundamental processes such as growth, development, and nutrient metabolism, as well as mediating adaptation to environmental stress. Their expression is closely associated with the development of insecticide resistance (Hatfield et al., 2016). This has been confirmed in studies involving chlorantraniliprole stress. For instance, treatment of *Bombyx mori* (Linnaeus, 1758), *Mythimna separata* (Walker, 1865), and *Grapholita molesta* (Busck, 1916) with chlorantraniliprole significantly upregulates *CarE* gene expression (Liu, Li, Yang, Chen, & Fan, 2022; Li et al., 2023; Shen, Duan, Ma, & Zhang, 2025). In addition, in *S. frugiperda*, exposure to chlorantraniliprole significantly increased *SfGSTe1* expression and was accompanied by improved sensitivity to the insecticide (Kong et al., 2025).

Despite the importance of *CarEs*, research on these genes in *G. mellonella* remains limited, the characteristics and expression patterns of *GmCarE4a* under insecticide

stress are unclear. In this study, we cloned the CDS of *GmCarE4a*, which encodes a carboxylesterase in *G. mellonella*, and conducted in-depth bioinformatics analysis. RT-qPCR was used to systematically analyze the expression patterns of this gene across different developmental stages, tissues, and under various chemical stress conditions. This study aims to contribute to a deeper understanding of the structure and expression patterns of carboxylesterases, and provides basic data on the detoxification mechanism of *CarE*. It will also provide potential molecular targets for the subsequent development of green control strategies enzyme inhibitors.

MATERIALS AND METHODS

Test Insect Source

Adults of *G. mellonella* were collected in August 2024 from the Miaojiang Bee Industry Apiary in Huangping County (107.66°E, 26.91°N), Guizhou Province, China, Specimens were transported to an artificial climate chamber (RXZ-380A, ,Jiangnan Instrument Factory, Ningbo) at the Guizhou Provincial Key Laboratory of Agricultural Biosafety for rearing. Meanwhile, honeycombs from the same apiary were maintained in circular plastic containers (10 cm diameter) until adult emergence. The emerged adults were placed in 10 cm-diameter containers without honeycombs, and a 10 cm oviposition strip was provided to facilitate egg laying. Rearing conditions were maintained at 30 ± 1 °C, 65% ± 5% relative humidity, and a photoperiod of L:D = 0:24 h until a stable experimental population was established (Wan, Li, Long, Smagghe, & Liu, 2025).

Total RNA Extraction and cDNA Synthesis

Total RNA was extracted from treated samples of fourth-instar larvae of *G. mellonella* using Trizol Up reagent (TransGen, ET111-01-V2, Beijing, China), and its integrity was assessed by electrophoresis on a 1% agarose gel. RNA concentration and purity were measured using a nucleic acid and protein analyzer (NanoDrop 2000, USA). First-strand cDNA was synthesized according to the instructions of the HiScript II Q RT SuperMix Kit (Vazyme, R323-01, Nanjing, China) and then stored at -20°C.

Cloning of the *GmCarE4a*

The *GmCarE4a* sequence was screened from the transcriptomic data of *G. mellonella* collected by our research group, and primers were designed using Primer Premier 5.0 software. Primer information is shown in Table 1. Using cDNA synthesized by the rapid synthesis method as a template, the CDS of *GmCarE4a* was amplified by PCR. The PCR reaction system (total volume 50 μL) consisted of template cDNA 2 μL, forward and reverse primers 2 μL each, 2 × Rapid Taq Plus Master Mix (Dye Plus) 25 μL, and ddH_2O 19 μL. The reaction procedure was as follows: pre-denaturation at 95°C for 5 min, denaturation at 95°C for 30 s, annealing at 55°C for 30 s, and extension at 72°C for 2 min, repeated for 34 cycles. The amplified products were subjected to 1% agarose gel electrophoresis. Purified DNA fragments were isolated using the HiPure Gel Pure DNA Mini Kit (Magen, D2111-02, Guangdong, China), ligated into the

pMD18-T vector, and transformed into *E. coli* DH5α competent cells. Positive clones were sent to Sangon Biotech Co., Ltd. (Shanghai, China) for sequencing. Sequence alignment was performed between the assembled sequences and the whole genome.

Table 1. Information on primers related to the *CarE4a.*

Primers	Primer sequences (5' - 3')	Purpose
GmCarE4a-F	GAGTAGTGATCGCATAAGGA	CDS amplification
GmCarE4a-R	CGAATCGAGATCTGAAGCTC	
q*GmCarE4a*-F	CACGATGGATCCCTCAGACG	Target gene amplification in real-time quantitative PCR
q*GmCarE4a*-R	GAGGGAGTCAGTGCTATCGC	
q*GAPDH*-F	GTCATTCCCGCACTTAATGG	Reference gene amplification in real-time quantitative PCR
q*GAPDH*-R	CAGCTTCCTTGACCTTCTGC	

Bioinformatics Analysis and Phylogenetic Tree Construction of *GmCarE4a*

The *GmCarE4a* sequence was submitted to the China National Center for Bioinformation (CNCB) and assigned a registration number. Concurrently, we performed bioinformatics analysis of the CDS and constructed a phylogenetic tree. The names and websites of the softwares used are shown in Table 2.

Table 2. Information of software used for bioinformatics analysis.

The name of the software	The analysis project	Website
NCBI	Search gene sequence	https://blast.ncbi.nlm.nih.gov/Blast.cgi
ORF finder	Open reading frame search	https://www.ncbi.nlm.nih.gov/gorf/orffinder
Scan Prosite	Conserved domain prediction	https://prosite.expasy.org/scanprosite/
Net NGlyc 1.0 server	Prediction of N-glycosylation sites	https://services.healthtech.dtu.dk/services/NetNGlyc-1.0/
NetOGlyc 4.0 Server	Prediction of O-glycosylation sites	https://services.healthtech.dtu.dk/services/NetOGlyc-4.0/
Protparam	Analysis of physical and chemical properties of proteins	https://www.expasy.org/resources/protparam
Protscale	The prediction of protein hydrophilicity distribution	https://www.expasy.org/resorces/protscale
TMHMM	The prediction of protein transmembrance structure	https://services.healthtech.dtu.dk/services/TMHMM/
SignalP6.0	The prediction of protein signal	http://www.cbs.dtu.dk/services/SignalP/
NetPhos3.1	Protein phosphorylation site predictio	https://services.healthtech.dtu.dk/services/NetPhos/
PSORT	Protein subcellular localization	https://www.genscript.com/psort.html
SOPMA	Prediction of protein secondary structure	https://npsa-prabi.ibcp.fr
SWISS-MODEL	Prediction of protein three-dimensional structure	http://swissmodel.expasy.org/
DNAMAN	Sequences alignment analysis	Local software
MEGA-X	Phylogenetic tree construct	Local software

RT-qPCR Analysis of *GmCarE4a*

Samples of *G. mellonella* were collected at various developmental stages, including eggs, first- to seventh-instar larvae, pupae, and adults. Different tissue samples were obtained by placing fourth-instar larvae on ice-cold wax trays and using dissecting tools to harvest the head, cuticle, fat body, foregut, midgut, hindgut, and Malpighian tubules. Fourth-instar larvae of *G. mellonella* were placed into beakers containing 20% chlorantraniliprole (FMC Corporation, Shanghai, China) LC_{30} at (18.079 mg L^{-1}) and LC_{50} (50.453 mg L^{-1}) concentrations, calculated from prior toxicity data (Wan, Li, Long, Smagghe, & Liu, 2024). Sterile water was used as the control. Each beaker contained 20 larvae. The larvae were exposed to the pesticide by contact for 10 seconds and then

removed. They were subsequently fed untreated feed, and samples were collected 72 h after insecticide exposure (based on pre-experimental data on gene expression peaks) (Wan, Li, Long, Smagghe, & Liu, 2024). Three biological replicates were set up. RT-qPCR was employed to detect the expression levels of *GmCarE4a* in the above samples, using glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*) as the housekeeping gene. The primers used are listed in Table 1. The procedure followed the instructions provided in the ChamQ Universal SYBR qPCR Master Mix kit (Vazyme, Q711-02, Nanjing, China). The RT-qPCR reaction system (20 μL), comprising 0.4 μL each of forward and reverse primers, 10 μL qPCR SuperMix (Q711-02), 8.2 μL ddH_2O, and 1 μL cDNA . The RT-qPCR reaction protocol consisted of 45 cycles: pre-denaturation at 95°C for 3 min, denaturation at 95°C for 10 s, annealing at 57°C for 15 s, and extension at 72°C for 20 s. Melting curve analysis was performed at the end. The experiment was conducted with three technical replicates.

Data Processing

Relative expression levels of *GmCarE4a* across developmental stages, tissues, and chlorantraniliprole concentrations were calculated using the $2^{\wedge\text{-}\Delta\Delta Ct}$ method (Yan, Liu, Li, Yang, & Xu, 2024). One-way ANOVA in SPSS 23.0 was used to analyze differences for statistical significance ($p < 0.05$). Data were graphed in GraphPad 10, and presented as mean ± standard error (Zhang et al., 2024).

RESULTS

Cloning and Sequence Analysis of *GmCarE4a*

The CDS of *GmCarE4a* from *G. mellonella* was amplified by RT-qPCR, with the CNCB accession OMIX013348. The open reading frame spanned 1743 bp and encoded 581 amino acids (Fig. 1). ScanProsite analysis identified a putative disulfide-bond-forming region at positions 122-132 (EDCLTINVYTP). Positions 219-234 contained the carboxylesterase B serine active site motif FGGDPNLVTITGCSAG. The catalytic triad comprised Ser_{232}, Glu_{361}, and His_{481}. centered on $Ser_{232.}$ Predicted substrate-binding residues were AGG (151-153), CSA (231-233), F (236), Y (386), NF (390-391), Y (430), H (482), and L (485). A signal peptide sequence (MFVNTLLICFLAACTLG) indicated the protein is likely secreted. Six N-linked glycosylation sites were predicted at positions 23-NKSA, 138-NCTK, 287-NNSK, 372-NDTL, 464-NHSF, and 499-NVSD. Four O-glycosylation sites were predictied by NetOGlyc 4.0 at S(52), NT(536-537), T(544), and T(546). The calculated molecular formula was $C_{2969}H_{4529}N_{783}O_{846}S_{23}$, with a relative molecular mass of 65.5 kDa, and a theoretical isoelectric point (pI) of 6.97, indicating near-neutral charge. Leucine and proline were most abundant at 8.8% and 7.6%, respectively. The sequence contained 59 negatively and 58 positively charged residues. The extinction coefficient was 86220, with a half-life of 30 h and the instability coefficient of 43.51 (>40), classifying the protein as unstable. The fat coefficient was 79.19, corresponding to an average hydrophilicity coefficient of -0.259 (Table 3). The most hydrophobic residue value was 3.033,and the least was -2.589, consistent with an overall hydrophilic character (Fig. 2). No transmembrane domains were predicted, indicating a

non-transmembrane protein. A total of 104 phosphorylation sites were predicted, including 38 threonine (T), 38 serine (S), and 28 tyrosine (Y) sites (Fig. 3). Subcellular localization was predicted as 33.4% endoplasmic reticulum, 22.2% extracellular (including cell wall), and 11.1% each in mitochondria, Golgi apparatus, vacuoles, and cytoplasm (Fig. 4).

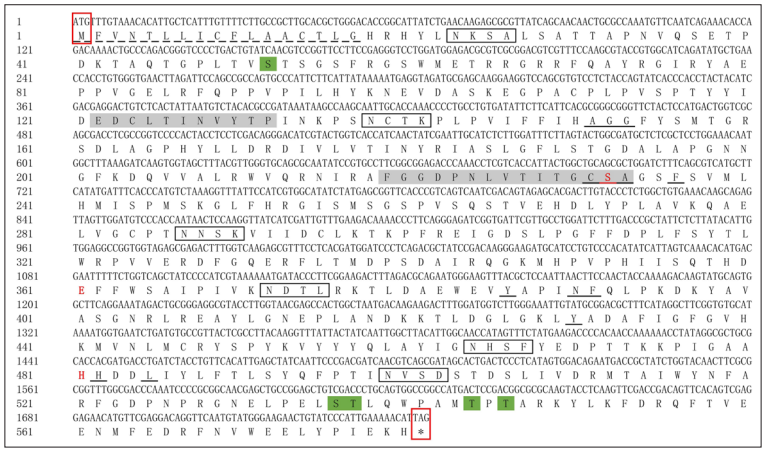

Figure 1. Nucleotide and deduced amino acid sequence of GmCarE4a. The initiation codon (ATG) and stop codon (TAG) are marked with red boxes. The gray shaded area represents the conservative amino acid sequence; the underlined part represents the substrate binding pocket; the part underlined with a dotted line represents the signal peptide sequence; the red font indicates the catalytic triad; the black rectangular box indicates the N-glycosylation sites; the green shading indicates the O-glycosylation sites.

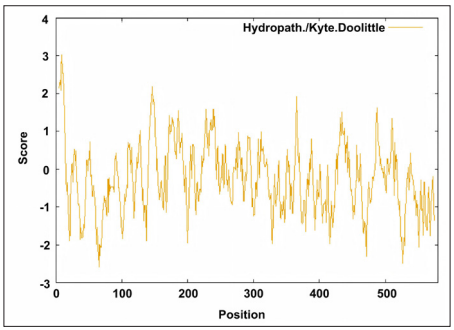

Figure 2. Visualization of the hydrophobicity of the GmCarE4a protein amino acids. Score > 0 indicates hydrophobicity; Score < 0 indicates hydrophilicity.

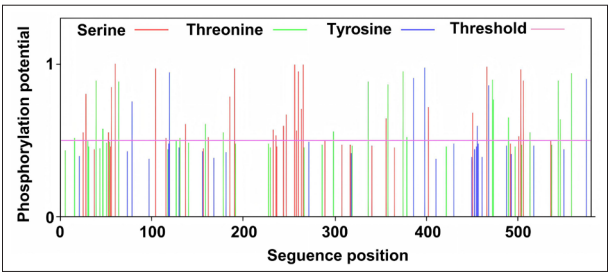

Figure 3. Prediction of phosphorylation sites of *G*mCarE4a.

Table 3. Physical and chemical properties of GmCarE4a.

Item	*GmCarE4a*
Protein molecular formula	$C_{2969}H_{4529}N_{783}O_{846}S_{23}$
Relative molecular mass (kDa)	65.5kDa
Theoretical isoelectric point (pI)	6.97
Ala content (%)	6.4
Arg content (%)	5.3
Asn content (%)	4.7
Asp content (%)	5.7
Cys content (%)	1.6
Gln content (%)	2.8
Glu content (%)	4.5
Gly content (%)	6.6
His content (%)	2.8
Ile content (%)	5.5
Leu content (%)	8.8
Lys content (%)	4.7
Met content (%)	2.4
Phe content (%)	5.7
Pro content (%)	7.6
Ser content (%)	6.6
Thr content (%)	6.6
Trp content (%)	1.4
Tyr content (%)	4.8
Val content (%)	5.9
Total number of negatively charged residues	59
Total number of positively charged residues	58
Extinction coefficient	86220
Half-life (h)	30
Instability coefficient	43.51
Fat coefficient	79.19
Average hydropathicity coefficient	-0.259

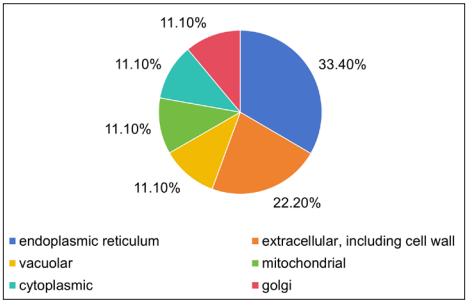

Figure 4. Proportion of CarE4a protein at different positions.

Protein Structure Analysis of *GmCarE4a*

Secondary structure was predicted with SOPMA. The *GmCarE4a* protein predominantly comprises random coils (60.86%) with α-helices at 33.45%, and extended strands at 5.69% (Fig. 5). Tertiary structure was modeled using SWISS-MODEL. A template-based model using SMTLID A0A6J1W716.1.A achieved a global model quality

score of 0.90, indicating a reliable prediction, the sequence identity to the template was 98.97% (Fig. 6a). In contrast, a model based on *Epiphyas postvittans* (7mp4.1.A) yielded a global model quality score of 0.67 (<0.8), indicating a comparatively unreliable structural prediction. The sequence identity to this template was 33.27% (Fig. 6b).

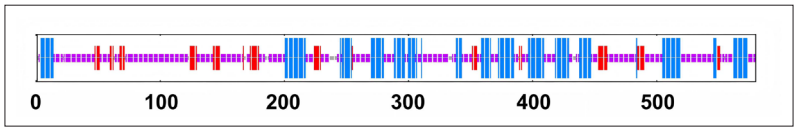

Figure 5. Prediction of secondary structure of GmCarE4a protein. Purple line: Irregular curling; Red line: Extended chain; Blue line: α-helix.

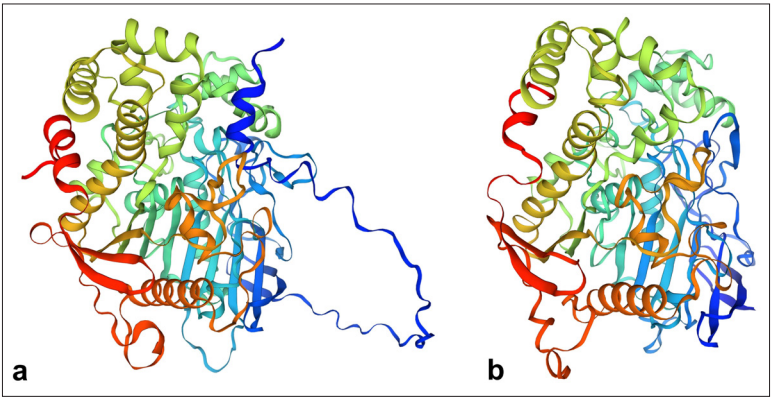

Figure 6. Prediction of the tertiary structure of GmCarE4a protein. a) represents *Galleria mellonella*, b) represents *Epiphyas postvittans*.

Multiple Sequence Alignment and Phylogenetic Analysis of *GmCarE4a*

Multiple sequence alignment between GmCarE4a from *G. mellonella* and homologs from *Achroia grisella* (Fabricius, 1794) (XP_059056064.1), *Streltzoviella insularis* (Staudinger, 1892) (QLI62115.1), *Ostrinia nubilalis* (Hübner, 1796) (XP_063834151.1), *Spodoptera exigua* (Hübner, 1808) (CAH0697269.1), *Plodia interpunctella* (Hübner, 1813) (XP_053620307.1), and *Amyelois transitella* (Walker, 1863) (XP_013194783.2) revealed amino acid sequence identities of 82.79%, 64.44%, 61.96%, 62.95%, 65.46%, and 64.95%, respectively (Fig. 7). The N-terminal regions near the active sites of insect CarEs were highly conserved, containing the catalytic triad as well as the disulfide-bond-forming motif EDCLTINVYTP and the serine active-site motif FGGDPNLVTITGCSAG.

A neighbour-joining (NJ) phylogenetic tree was constructed using GmCarE4a amino acid sequences (Fig. 8). Lepidoptera, Coleoptera, Diptera, and Hemiptera each form distinct clusters, indicating that CarE4a amino acid sequences within the same order were more similar due to shared phylogeny. *G. mellonella* GmCarE4a clustered within the Lepidoptera clade and formed a separate branch with *A. grisella* with 100% bootstrap support, indicating a close phylogenetic relationship.

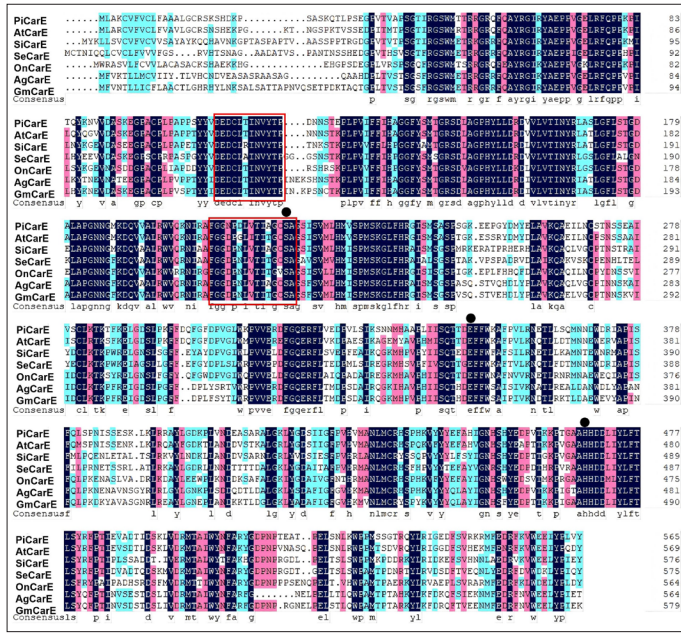

Figure 7. Comparison of the amino acid sequence of the *G.mellonella* CarE protein with the sequences of other species. Note: The black blue base similarity is 100%; the purple-red background base similarity is 100% to 75%; and the light blue background base similarity is 75% to 50%. The sequence information were from NCBI (AgCarE: *A. grisella*XP_059056064.1, SiCarE: *S. insularis* QLI62115.1, OnCarE: *O. nubilalis* XP_063834151.1, SeCarE: *S. exigua* CAH0697269.1, PiCarE: *P. interpunctella* XP_053620307.1, AtCarE: *A. transitella* XP_013194783.2). The catalytic triad is indicated by a circle, while the conserved amino acid sequence structure is indicated by a rectangle.

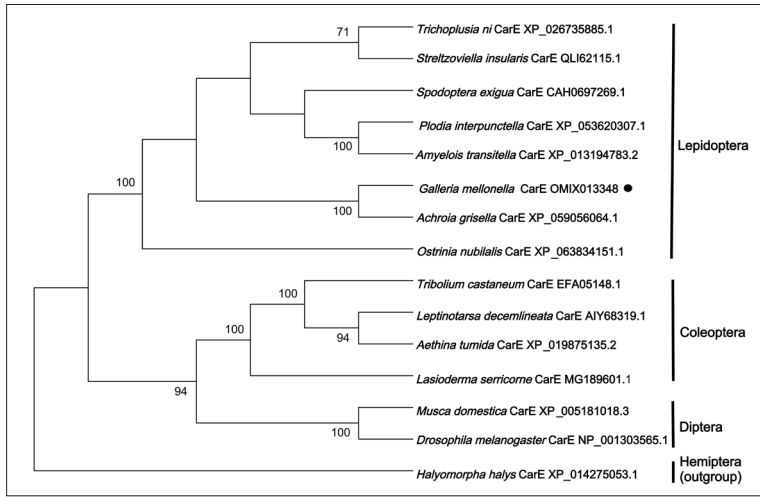

Figure 8. Phylogenetic analysis of insect CarE4a based on amino acid sequence. The values at the branch points represent the confidence of bootstrap values calculated from 1000 clustering iterations, with the branches labeled as the CarE4a.

Expression Analysis of *GmCarE4a*

RT-qPCR analysis indicated that *GmCarE4a* expression in *G. mellonella* varies across developmental stages (Fig. 9). *GmCarE4a* was detected in eggs, larvae, pupae, and adults, with expression in larval significantly higher than in other stages (larva vs. egg/pupa/female/male: $p < 0.05$). With the larval stage, except for the 7th- instar and the 3rd-instar, the 6th-instar expression was significantly higher than in most other instars (L_6 vs. $L_1/L_2/L_4/L_5$: $p < 0.05$). First-, third-, and 7th-instar larvae showed intermediate expression levels that were higher than those of second-, fourth-, and fifth-instar larvae ($L_1/L_3/L_7$ vs. $L_2/L_4/L_5$: $p < 0.05$). Second- and fourth-instar larvae exhibited intermediate expression, whereas 5th-instar larvae showed the lowest expression, significant lower than all other instars (L_5 vs. $L_1/L_2/L_3/L_4/L_6/L_7$: $p < 0.05$). No significant difference between was observed between female and male adults (female/male: $p > 0.05$). (Fig. 9).

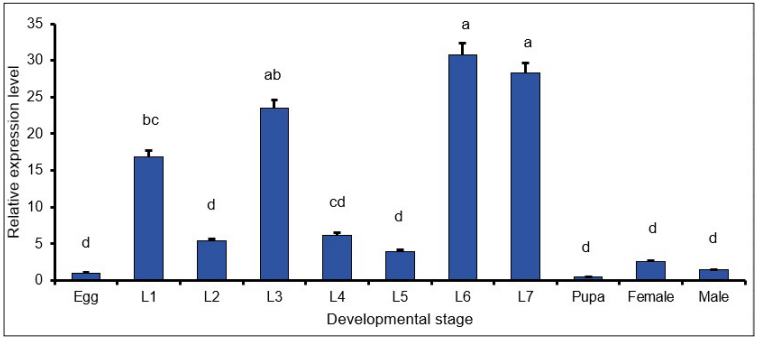

Figure 9. Expression profiles of *GmCarE4a* in *G. mellonella* at different developmental stages.

The relative expression levels of *GmCarE4a* differed across various tissues in the 4th-instar of *G. mellonella* larvae (Fig. 10). The epidermis exhibited significantly higher expression levels compared to other tissues (epidermis vs. head/foregut/midgut/hindgut/Malpighian tubes/fat body: $p < 0.05$). Hindgut expression was secondary but significantly lower than in the epidermis (hindgut vs. epidermis: $p < 0.05$). Expression in the foregut, midgut, Malpighian tubules, and fat body was intermediate, whereas the head exhibited the lowest expression, significantly lower than in other tissues (head vs. foregut/midgut/hindgut/Malpighian tubes/fat body/epidermis: $p < 0.05$) (Fig. 10).

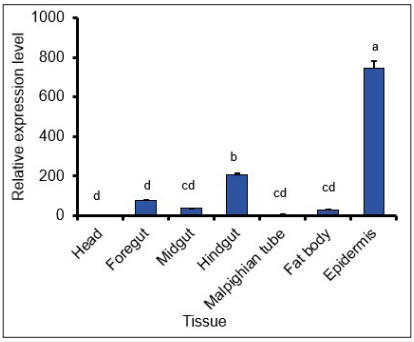

Figure 10. Expression profiles of *GmCarE4a* in *G. mellonella* at different tissues

In 4th-instar larvae, *GmCarE4a* expression varied significant across chlorantraniliprole concentrations (CK, LC_{10}, LC_{30}, and LC_{50}) (Fig. 11). *GmCarE4a* expression peaked at LC_{50}, followed by CK, and showed no significant increase compared to LC_{10} and LC_{30} (LC_{50} vs. LC_{10}/LC_{30}: $p < 0.05$; CK vs. LC_{10}/LC_{30}: $p < 0.05$). Under LC_{10} conditions, the *GmCarE4a* exhibited the lowest expression levels compared to other concentrations (LC_{10} vs. CK/LC_{30}/LC_{50}: $p < 0.05$) (Fig. 11).

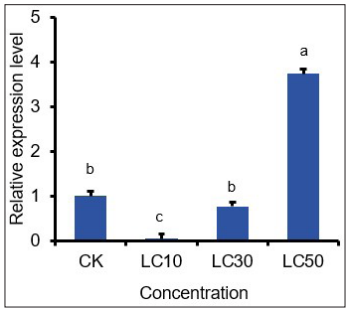

Figure 11. Expression profiles of *GmCarE4a* in *G. mellonella* under chlorantraniliprole stress

DISCUSSION

This study obtained the CDS of *GmCarE4a* from *G. mellonella*, which encoded a protein of 65.5 kDa. The amino acid sequence of *GmCarE4a* contained a disulfide-bond-forming motif at positions 122-132 (EDCLTINVYTP) and a carboxylesterase B-type serine active site at positions 219-234 (FGGDPNLVTITGCSAG). These features indicated the presence of a substrate-binding site and a catalytic triplet S-E-H in GmCarE4a amino acid sequence. These structures were crucial for maintaining higher-order structural integrity and protein stabilizing catalytic activity (Schrag, Li, Wu, & Cygler, 1991). The signal peptide region was found to be within the first 17 N-terminal amino acid residues at the 5′ end, suggesting that GmCarE4a was likely secreted. Glycosylation-site prediction indentified six N-linked glycosylation sites, and N-glycosylation might contribute to protein stability and the conformation of the enzyme active site (Wheelock, Shan, & Ottea, 2005). It could be inferred that GmCarE4a possesses not only a stable structure but also highly efficient catalytic activity, enabling it to metabolize and detoxify certain toxic substances.

GmCarE4a exhibited significant differences in its spatiotemporal expression patterns in *G. mellonella*. During the developmental stages, the larval stage expression level was significantly higher than that at other stages, likely reflecting increased feeding and a corresponding need for elevated detoxification capacity. Similar larval-biased expression has been reported in *Plutella xylostella* (Linnaeus, 1767) (Ren et al., 2015). At the tissue, expression levels were significantly higher in epidermal and hindgut than in other tissue, suggesting that these tissues perform critical physiological functions within the insect body. This pattern aligns with reports of high *SlCarE054* expression in digestive tissues of *Spodoptera litura* (Fabricius, 1775) (Xu et al., 2024). This expression difference suggested that *GmCarE4a* contributes to larval growth and development, detoxification and defense, and the maintenance of normal physiological functions in *G. mellonella*.

Investigating carboxylesterase expression under pesticidal stress helped clarify its role in insecticides metabolism (Shen, Duan, Ma, & Zhang, 2025). Under exogenous chemical stress, insects activated their internal defense and detoxification pathways, mounting physiological responses to counteract toxic effects (Wan Li, Long, Smagghe, & Liu, 2025; Lin et al., 2022). In this study, as chlorantraniliprole concentration increased, *GmCarE4a* expression in *G. mellonella* reached a peak. This pattern aligned with findings by Ren et al. (2023), who reported an upward trend in *CarE* expression in *Galeruca daurica* (Joannis, 1865) following chlorantraniliprole stress. Similarly, chlorantraniliprole treatment significantly increased *MsCarE3* and *MsCarE4* expression in fourth-instar *M. separata* larvae (Liu, Li, Yang, Chen, & Fan, 2022). In resistant third-instar *P. xylostella* chlorantraniliprole exposure also upregulated *pxCCE016b* expression (Hu et al., 2016). Based on the findings of this study, it was speculated that *GmCarE4a* may be involved in the detoxification and metabolic processeed of *G. mellonella* in response to the chlorantraniliprole. Across concentrations, *G. mellonella* enhanced its detoxification capacity by upregulating the expression of *GmCarE4a*. These insights will be important for understanding insecticide-insect interactions and resistance mechanisms and provide a theoretical basis for the rational use of insecticides in agricultural production.

This study successfully cloned the CDS of *GmCarE4a* from *G. mellonella*, which was 1743 bp and encoded 581 amino acids. The predicted protein was classified as a β-esterase. *GmCarE4a* showed its highest expression levels in mature larvae and in epidermal tissues, and its expression increased significantly under insecticide stress, suggesting a key role in pesticides detoxification and metabolism in *G. mellonella*. This study enriched the fundamental knowledge of detoxification and metabolic genes in *G. mellonella*, and subsequent functional studies can be performed using RNA interference (RNAi). This study will provide a theoretical basis for elucidating the molecular pathways by which carboxylesterases contribute to the evolution of insecticide resistance and offers data to inform the optimization of pest management strategies targeting resistance genes in *G. mellonella*.

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