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ORIGINAL ARTICLE

Transcriptome analysis reveals glycometabolism and antioxidation-related genes involved in the antifungal immune response of *Spodoptera frugiperda* larvae against *Beauveria bassiana* infection

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Abstract. *Spodoptera frugiperda* damages crops around the world and has developed resistance to many pesticides. *Beauveria bassiana*, a fungus that is harmless to humans and the environment, is widely used in pest control. In our study, differentially expressed genes between *S. frugiperda* larvae, both exposed and unexposed, to *B. bassiana* were analyzed by transcriptome sequencing. More than 160 Gb of clean data were obtained, and 2767 and 2892 DEGs were identified in LH36vsCK36 and LH144vsCK144, respectively. To explore the roles of glycometabolism and antioxidation-related enzyme genes in *S. frugiperda* against *B. bassiana* infection, the expression patterns of those genes when under attack from *B. bassiana* were analyzed by quantitative real-time PCR. The results of enzyme activity experiments revealed that *S. frugiperda* larvae exposed to *B. bassiana* could upregulate these genes to produce more enzymes related to the maintainance of normal glucose metabolism, as well as regulate the expression of detoxification and antioxidant factors to enhance the larvae's detoxification and antioxidant capacity. The result implied that glycometabolism and antioxidation-related enzymes and genes played critical roles in the antifungal immune process of *S. frugiperda* larvae. This study enhances our understanding of the molecular mechanisms related to regulation of metabolism and provides a basis for exploring new methods to combat antifungal resistance in *S. frugiperda*.

INTRODUCTION

In natural ecological regulation, entomopathogenic fungi are an important external control factor that can mediate the spread of insect epidemics and lead to dramatic declines in host populations. Due to the insecticidal activity of these fungi, a variety of entomopathogenic fungi have been successfully used as a powerful alternative to chemical pesticides (Hong et al., 2024). Fungi are also the most common cause of insect disease in nature. It has been reported that around 1000 species of fungus can kill insects, spiders or mites, and are particularly suitable for the development and utilization of biogenic pesticides (Li et al., 2010). Unlike bacterial and viral infections, entomopathogenic fungi infect the host mainly by direct penetration of the insect epidermis and can kill insects through contact (Jabbour et al., 2011; Wang & Feng, 2014). Normally, fungal infection begins with the attachment of conidia, a large number of spores are attached to the insect epidermis, catalytically producing active enzymes and other related secretions that promote epidermal osmosis. Under the action of these substances, fungi form appressoria and pierce the body wall of insects with mycelium. After the growing mycelium penetrates the body cavity, it propagates by relying on the internal nutrients of the infected insects (Jabbour et al., 2011). Once the nutrition of the insect body is exhausted, the mycelium reproduces, producing new conidia and releasing them into the environment, thereby starting a new infection cycle (Jabbour et al., 2011). Among them, *Beauveria bassiana* is the most mature in terms of commercial application, and it is also one of the model species employed to study the infection of pathogenic microorganisms on insects (Wang et al., 2021). *B. bassiana* is a kind of broad-spectrum entomopathogenic fungus belonging to the genus *Beauveria.* It is widely distributed in nature. *B. bassiana* mainly produces a large number of conidia, which germinate on the surface of the insect body. After infecting the insect body, conidia take the blood cells and other tissue cells in the insect body as nutrients to supply

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their reproduction. Then, they infest the insect body with mycelium and produce white powdery conidia, making the infected insect appear white and stiff (Mascarin & Jaronski, 2016).

Insects produce a large number of oxygen-containing free radicals in the metabolic process, including anion radicals O^{2-}, OH^{-}, and peroxide radicals RO^{-} and ROO^{-}, which are called reactive oxygen species (ROS) (Dalton et al., 1999). The accumulation of reactive oxygen species can cause damage to cell DNA and mitochondria. Insects have a reactive oxygen scavenging system, which is mainly controlled and regulated by three protective enzymes, superoxide dismutase (SOD), peroxidase (POD) and catalase (CAT) (Sezer & Ozalp, 2015; Jia et al., 2016). Under normal conditions, the antioxidant reaction catalyzed by the three enzymes can regulate the dynamic balance of reactive oxygen species in insects and maintain the stability of the physiological and biochemical environment in insects (Sezer & Ozalp, 2015; Jia et al., 2016). After the insecticidal fungi such as *B. bassiana* infect the insects, the reactive oxygen species balance will be broken and the activity of antioxidant enzymes will change accordingly, which is also a kind of insect autoimmunity. Superoxide dismutase (SOD) is widely found in organisms and is the first line of defence for removing reactive oxygen species (Stephenie et al., 2020). SOD plays an important role in the resistance to stress of organisms. Superoxide anion (O^{2-}) can be dismutated by this enzyme to produce oxygen (O_2) and hydrogen peroxide (H_2O_2) (Maurya & Namdeo, 2022). Superoxide dismutase (SOD) is a kind of enzyme containing active centres of metal elements, mainly including Cu/Zn-SOD, Mn-SOD, Fe-SOD and Ni-SOD (Zeinali et al., 2015). Catalyzed by CAT and POD, superoxide ions generate H_2O_2 to reduce the damage to the organisms (Nyathi & Baker, 2006; Rhee, 2006; DeJong et al., 2007). CAT is a terminal oxidase with an iron porphyrin ring as a prosthetic group, which can be divided into eukaryotic CAT and prokaryotic CAT according to its source. In eukaryotes, CAT mainly exists in organelles, while prokaryotic CAT exists in most aerobic microorganisms. According to their sequence differences, peroxidase can be divided into two superfamilies, one derived from animals and the other from bacteria, fungi and plants (Welinder, 1992). The antioxidant system of different insects also stimulated different responses to *B. bassiana*.

Dehydrogenase is an important part of the REDOX enzyme system, widely distributed in animals, plants and microorganisms, where it plays a key role in physiological activities such as oxidative processes. Malate dehydrogenase (MDH) catalyzes the reduction of oxaloacetate to L-malate in the tricarboxylic acid (TCA) cycle (Shimozawa et al., 2022). Lactate dehydrogenase (LDH) is an enzyme that catalyzes the conversion of pyruvate, the end product of glycolysis, into lactate (and vice versa) with concomitant interconversion of NADH and NAD^{+} (Long et al., 2020; Xia et al., 2011). Isocitrate dehydrogenase (IDH), a mitochondrial enzyme of the tricarboxylic acid cycle, catalyzes the formation of α-ketoglutaric acid from isocitrate (Duncan et al., 2017). Pyruvate dehydrogenase (PDH) is the rate-limiting enzyme that connects pyruvate to the TCA cycle and to oxidative phosphorylation (Patel et al., 2014). Succinate dehydrogenase (SDH) converts sorbitol into fructose and plays an important role in the sorbitol metabolic pathway (Rubio et al., 2011; Wang et al., 2013). After infection with *B. bassiana*, the activity and expression levels of lactate dehydrogenase and malate dehydrogenase in the insect's body will be changed, resulting in physiological and metabolic disorders of cells (Tian et al., 2008).

Spodoptera frugiperda (Smith, 1997) (Lepidoptera: Noctuidae), originating from the Americas, is one of the most dangerous agricultural pests in the world and causes serious damage to economic crops every year (Todd & Poole, 1980; Martinelli et al., 2006). The physiological characteristics and development of resistance in *S. frugiperda* have made the reuse and overuse of pesticides become a potential risk to human and environmental health problems. The abuse of chemical pesticides has caused many problems, including drug resistance and environmental pollution. In contrast to conventional chemical control, the use of *B. Bassiana* as a method of microbial control is non-toxic to humans, livestock and the environment, while exhibiting strong virulence against pests (Ramos et al., 2017).

To date, there have been few studies on the effects *B. bassiana* infection on antioxidant and carbohydrate metabolism genes, as well as enzyme activity, in *S. frugiperda*. Therefore, in our study, high-throughput transcriptome sequencing was performed on *S. frugiperda* infected 36 h and 144 h after *B. bassiana* infection, respectively, to find out the genes of antioxidant enzyme and carbohydrate metabolic enzymes of *S. frugiperda* to *B. bassiana*. This study will offer potential strategies for enhancing the control effectiveness of *B. bassiana* against *S. frugiperda*, and advance our understanding of the resistance mechanisms of *S. frugiperda* to *B. bassiana*.

MATERIAL AND METHODS

Insects and tested *B. bassiana* strains

The experimental subjects for this study were third instar larvae of *S. frugiperda*, reared at the Institute of Nanfan & Seed Industry, Guangdong Academy of Sciences, Guangzhou, China. The larvae were maintained under controlled conditions of 27 ± 1°C temperature, 65%~85% relative humidity, and a 14L : 10D photoperiod. To ensure consistency, larvae with identical individual length and instar were selected after three generations.

The *B. bassiana* strain BbHN6, which was obtained from the Institute of Nanfan & Seed Industry, Guangdong Academy of Sciences, Guangzhou, China, was utilized in this study. BbHN6 was cultivated on potato dextrose agar (PDA) for a duration of 14 days at a temperature of 25°C. 10 mL of 0.15 mol/L NaCl solution (containing 0.05% Tween-80) was added to the BbHN6 plate and thoroughly agitated to wash off spores. The mixture of spores and hyphae was transferred to a 50 mL centrifuge tube, and then the mixture was swirled for 10 min to disperse the spores and filtered through a sterilized cotton ball to obtain a single spore suspension of *B. bassiana*.

Spore suspensions of *B. bassiana* in the range of 1.0×10^4 to 1.0×10^8 spores /mL were set to test their pathogenicity to *S. frugiperda* larvae. Dead individuals first appeared on the third day.

The mortality rate of larvae increased with increased spore concentration and increased time since exposure. The cumulative mortality rate reached the highest (over 85%) on the 7th day after exposure. The concentration (1.0×10^8 spores /mL) of spore suspension of *B. bassiana* was chosen to ensure that the majority of the larvae in the experimental group could be infected (mycelial growth after death was checked to confirm the success of the protocol) (Fig. S1A and B). The larvae of *S. frugiperda* were soaked in the spore suspension of *B. bassiana* (1×10^8/mL) for 10 s, then placed on sterile filter paper to absorb excess water, while a control group was treated with a 0.05% Tween-80 solution. All the larvae were transferred to separate compartment feeder boxes and fed with fresh corn every day, and were covered with two layers of paper towels sprayed with sterilized water for moisturization with controlled conditions of 27 ± 1°C temperature, 65%~85% relative humidity, and a 14L : 10D photoperiod. The larvae exposed to *B. bassiana* were collected at specific time intervals of 12 h, 24 h, 36 h, 48 h, 60 h, 72 h, and 144 h post-treatment. Subsequently, the larvae were rapidly frozen using liquid nitrogen and stored at a temperature of –80°C.

cDNA library construction and transcriptome sequencing

The larvae of *S. frugiperda*, which were exposed to *B. bassiana* and collected at two different time points, 36 h and 144 h post-treatment, were utilized for the extraction of RNA (LH36vsCK36 and LH144vsCK144 were used for transcriptome data analysis. LH36vsCK36 refers to *S. frugiperda* larvae 36 h after exposure and unexposure to *B. bassiana*, and LH144vsCK144 refers to *S. frugiperda* larvae 144 h after exposure and unexposure to *B. bassiana*). This was conducted with three biological replicates per treatment group. The sample was placed in a precooled mortar, liquid nitrogen was added, and the sample was quickly ground to a powder with a grinding pestle. The powder was transfered to a 1.5 mL RNase-free centrifuge tube with 500 µL of Trizol solution (Invitrogen, USA), mixed thoroughly, and sustained at room temperature for 5 min. 200 mL of chloroform was added to the centrifuge tube, mixed by shaking, and left to stand at room temperature for 5 min, before being centrifuged for 15 min at 4°C, 12,000 r/min. The supernatant was collected after centrifugation, and transferred to a new 1.5 mL RNase-free centrifuge tube with 500 µL of pre-chilled isopropanol solution, inverted gently to mix, and incubated at 4°C for 20–30 min. It was then centrifuged at 4°C, 12,000 rpm for 10 min, the supernatant discarded, and the obtained RNA precipitate is visible at the bottom of the centrifuge tube. 1 mL of 75% ethanol was added to the centrifuge tube, inverted several times to suspend the sediment at the bottom for washing. The samples were then centrifuged at 7500 r/min for 5 min at 4°C and the supernatant was discarded. The above steps were repeated and the sediment was dried at room temperature on a ultra-clean bench until it became translucent. Finally, 50 mL of DEPC-treated H_2O was added to the centrifuge tube to dissolve the RNA sample and it was stored at –80°C.

The quality and integrity of the RNA were assessed using agarose gel electrophoresis and the Agilent Bioanalyzer 2,100 system (Agilent Technologies Inc., located in CA, USA). The purity and concentration of the RNA were evaluated using a Nanodrop ND2000 spectrophotometer (NanoDrop Technologies Inc., based in Wilmington, DE). The construction of the cDNA library was performed in line with Illumina's sample preparation instructions (Illumina, San Diego, CA). The library was sequenced on the Illumina HiSeq™ 4,000 platform (Illumina, San Diego, CA, USA), resulting in obtaining paired-end reads of 150 bp. This process was carried out at Novogene Bioinformatics Technology Co. Ltd (Beijing, China).

De novo contig assembly and functional annotation

Clean reads were generated from raw data after adaptors, primers, ambiguous 'N' nucleotides, and low-quality sequences (50% of bases had a quality value of 5) were removed. Q30 (the percentage of bases with a Phred value of at least 30) and GC content were used to assess the quality of the clean reads, which were assembled into contigs using Grabhe's Trinity software (Grabherr et al., 2011). After transcripts were gathered, they were assembled using the De Bruijn graph method, and unigenes were obtained using TGI Clustering Tool (created by Pertea et al., 2003 and Quackenbush et al., 2001).

Unigene annotation and function classification

A threshold of 10^{-5} E-values was applied to each unigene obtained, for alignment against known protein sequences in the NCBI Nonredundant (NCBI-NR), UniProt, Kyoto Encyclopedia of Genes and Genomes (KEGG), and Gene Ontology (GO) databases. A GO analysis was performed on each unigene using Blast2GO in conjunction with WEGO software (Ye et al., 2006). Metabolic pathway annotations were determined based on KEGG annotations (Kanehisa et al., 2008).

Expression analysis by real-time quantitative PCR (qRT-PCR)

The expression of the target genes was conducted via real-time quantitative PCR (qRT-PCR). Tissue samples were obtained from *S. frugiperda* larvae exposed to *B. bassiana* at various time points after treatment (12 h, 24 h, 36 h, 48 h, 60 h, 72 h, and 144 h) with three biological replicates. The first-strand cDNA was synthesized from total RNA extracted from these samples according to the manufacturer's instructions (provided by the PrimeScript™ RT Reagent Kit, provided by Takara, Japan). qRT-PCR was conducted in 10 µL reaction volumes [1 µL cDNA (2 ng/µL), 5 µL SYBR Green I Master (Roche Diagnostics Ltd., Lewes, UK), 0.5 µL/primer, and 3 µL ddH_2O] on a LightCycler® 480 real-time PCR system (Roche Diagnostics Ltd) with the following program: denaturation at 95°C for 5 min followed by 40 cycles of 5 s at 95°C, 20 s at 60°C, and 20 s at 72°C. *gapdh* was used as the internal reference gene for testing each gene in triplicate. The relative expression levels of the candidate glycometabolism and antioxidation-related enzyme genes normalized to the internal control gene were calculated using the $2^{-\Delta\Delta Ct}$ method (Livak & Schmittgen, 2001).

Assays of the activity of enzymes associated with antioxidation and glycometabolism in *S. frugiperda* after exposure to *B. bassiana*

Larvae after exposure to *B. bassiana* and unexposed larvae of *S. frugiperda* were collected as samples, 1 mL of precooled 0.1 M pH 7.0 phosphate buffer was added, the homogenate was conducted in an ice bath, and then centrifuged with 1000 rpm for 10 min at 4°C, and the supernatant was retained for subsequent experiments. The activity of glutathione peroxidase (GSH-PX), SOD, CAT, glutathione-S-transferase (GST), IDH, LDH, MDH, PDH and SDH of each sample was detected according to the instructions of reagent kits (Nanjing Jiancheng, China) (Solarbio, Beijing Solarbio Science & Technology Co., Ltd, China). Three replicates were tested for each sample, and U/mg or U/mgprot were used to indicate the activity of different enzymes related to antioxidation and glycometabolism in each sample, depending on the different reagent kit and method used.

Data analyses

All results are expressed as the mean ± SEM, and the data were analysed using one-way analysis of variance (ANOVA), followed

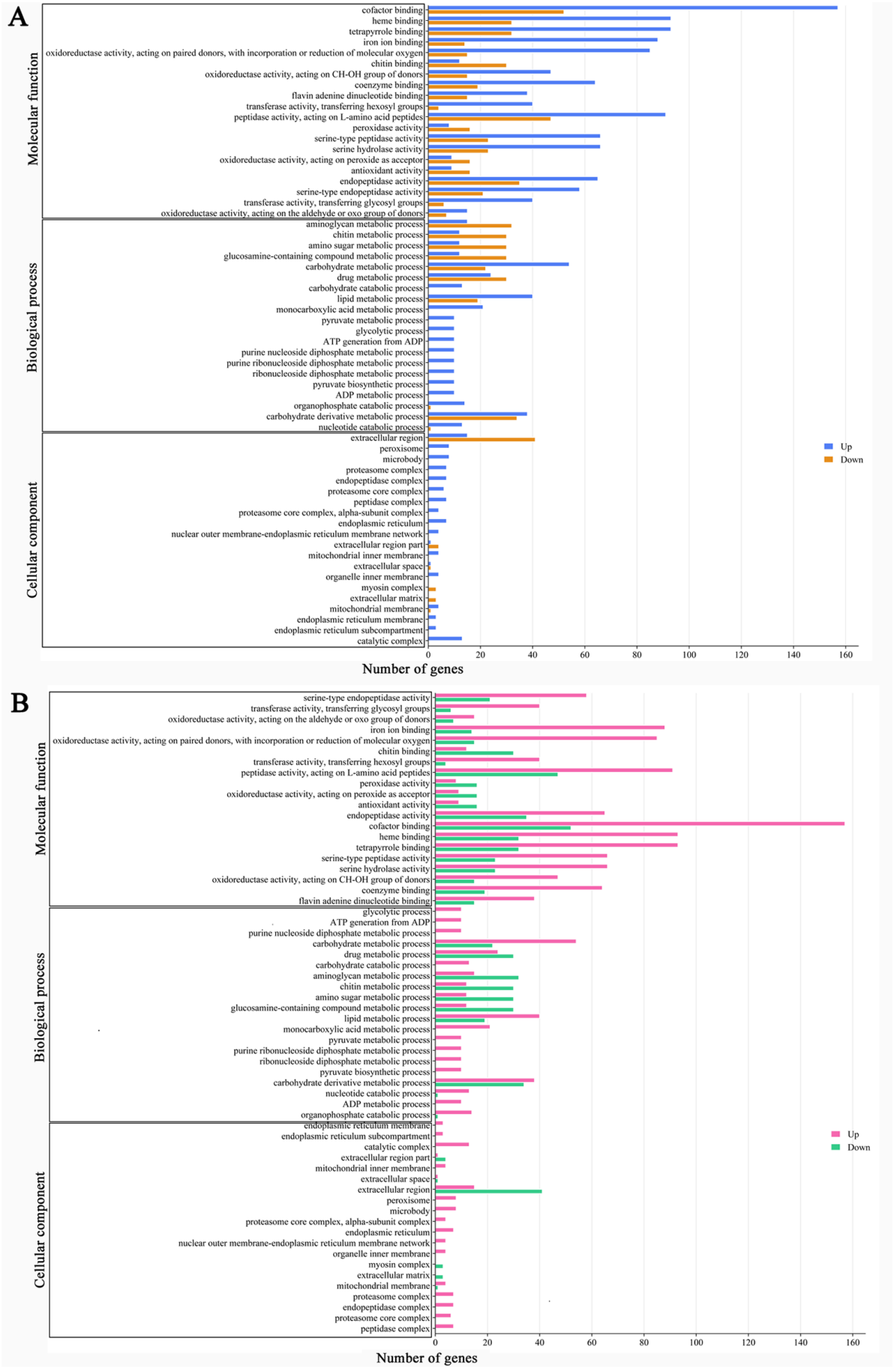

Fig. 1. Secondary GO annotation statistical of DEGs. (A) The top functional enrichment of DEGs in biological process, cellular component and molecular function in LH36vsCK36; (B) The top functional enrichment of DEGs in biological process, cellular component and molecular function in LH144vsCK144.

by Duncan's multiple range test for multiple comparisons. Statistical analyses were performed using the SPSS 19.0 statistical software package.

Data accessibility

The Illumina reads of *S. frugiperda* were submitted to the NCBI Short Archive (SRA) with Bioproject PRJNA987447. Their accession numbers are SAMN35982286, SAMN35982287, SAMN35982288 and SAMN35982289.

RESULTS

Overview of the transcriptome data analysis of *S. frugiperda* larvae exposed to *B. bassiana*

The transcriptome sequencing data for the comparisons LH36vsCK36 and LH144vsCK144 of *S. frugiperda* larvae exposed to *B. bassiana* were generated using the Illumina HiSeq 4,000 platform. This resulted in the production of 1,155,833,496 raw reads and 1,111,415,334 clean reads, respectively. Following the quality control process, over 160 Gb of clean data were obtained from the 12 libraries. The Q30 and GC content of each library were over 92.52% and 43.95%, respectively, as shown in Table 1. The unigenes obtained from the transcriptome sequencing data were annotated in the National Center for Biotechnology Information Non-Redundant Proteins (NCBI-NR), Gene Ontology (GO), and Kyoto Encyclopedia of Genes and Genomes (KEGG) databases.

Differential gene expression analysis and functional annotation

There were 2767 and 2892 DEGs in LH36vsCK36 and LH144vsCK144 based on statistical analysis, respectively. These DEGs included 1541 up-regulated and 1226 down-regulated genes in LH36vsCK36 as well as 1261 up-regulated and 1631 down-regulated genes in LH144vsCK144. In LH36vsCK36 and LH144vsCK144, annotated DEGs with functional groups were under three main GO categories ("biological process", "cellular component" and "molecular function") via Blast2GO and WEGO software, respectively (Fig. 1). In LH36vsCK36, the analysis of up-regulated DEGs showed that "carbohydrate metabolic process" and "lipid metabolic process" were predominant terms in the "biological process" category; In the "cellular component" category, "extracellular region" is the most abundant term; In the "molecular function", "cofactor binding" was predominant term, followed by "heme binding" and "iron ion binding" terms. The analysis of down-regulated DEGs revealed that "aminoglycan metabolic process", "amino sugar metabolic process", "chitin metabolic process", and "drug metabolic process" were the most abundant terms in the "biological process" category; In the "cellular component" category, "extracellular region" was predominant term; In the "molecular function", "cofactor binding" was predominant term, followed by "heme binding", "chitin binding" and "tetrapyrrole binding" (Fig. 1A). In LH144vsCK144, the analysis of up- and down-regulated DEGs all showed that "proteolysis", "extracellular region" and "cofactor binding" were predominant terms in the "biological process", "cellular component" and "molecular function" categories, respectively (Fig. 1B).

From the KEGG enrichment analysis, the most significant 20 KEGG pathways were listed. In LH36vsCK36, the DEGs were mainly enriched in "Biosynthesis of cofactors" and "Carbon metabolism", followed by "Fatty acid metabolism", "Metabolism of xenobiotics by cytochrome P450" and "Drug metabolism- cytochrome P450"; While, in LH144vsCK144, the DEGs were mainly enriched in "Biosynthesis of cofactors", "Lysosome" and "Drug metabolism-other enzymes", followed by "Drug metabolism- cytochrome P450", "Metabolism of xenobiotics by cytochrome P450" and "Retinol metabolism" (Fig. 2).

Validation of DEG data by qRT- PCR

To further validate the transcriptome results in the comparisons of LH36vsCK36 and LH144vsCK144, ten differentially expressed genes (DEGs) were randomly selected for quantitative real-time PCR (qPCR) analysis. *gapdh* was employed as the internal reference gene for statistical analysis of the DEGs. The expression data were presented as $\log_2$ values of fold changes in gene expression, with *gapdh* used as the reference for normalization. Despite some discrepancies in the fold changes, the overall expression tendency was consistent with that of the DEGs data, as shown in Fig. 3. This finding suggests that the DGE results obtained from the transcriptome analysis are reliable.

Expression patterns of factors related to antioxidation in *S. frugiperda* infected with *B. bassiana*

The result of qPCR revealed that after exposure to *B. bassiana*, the relative expression of *Sfrusod* was up-regulated in the early stage of exposure from 12 to 36 h, and then showed a down-regulated trend (Fig. 4); the relative expression of *Sfrucat* was firstly slightly down-regulated from 12 to 24 h, and sharply up-regulated 36 h after exposure, then gradually down-regulated from 48 to 144 h (Fig. 4); the peaks of relative expression of *Sfrugsh-px6* and *Sfrugst2* appeared at 48 h and 24 h after exposure, and then they all showed a down-regulated trend (Fig. 4); while the relative expression of *Sfrugstd7* presents an undulating, wave-like variation (Fig. 4).

Table 1. Summary of transcriptome of LH36vsCK36 and LH144vsCK144.

Sample	Library	Totally raw reads	Totally clean reads	Q30	GC_pct (%)
LH36_1	FRAS210259905-1r	1155833496	1111415334	93.33	48.15
LH36_2	FRAS210259906-1r			93.20	46.61
LH36_3	FRAS210259907-1r			93.43	45.84
LH144_1	FRAS210259908-1r			93.01	45.82
LH144_2	FRAS210259909-1r			93.63	44.24
LH144_3	FRAS210259910-1r			93.12	43.95
CK36_1	FRAS210259911-1r			92.52	44.32
CK36_2	FRAS210259912-1r			92.71	48.01
CK36_3	FRAS210259913-1r			93.37	47.7
CK144_1	FRAS210259914-1r			93.03	47.41
CK144_2	FRAS210259915-1r			93.16	47.88
CK144_3	FRAS210259916-1r			93.38	46.85

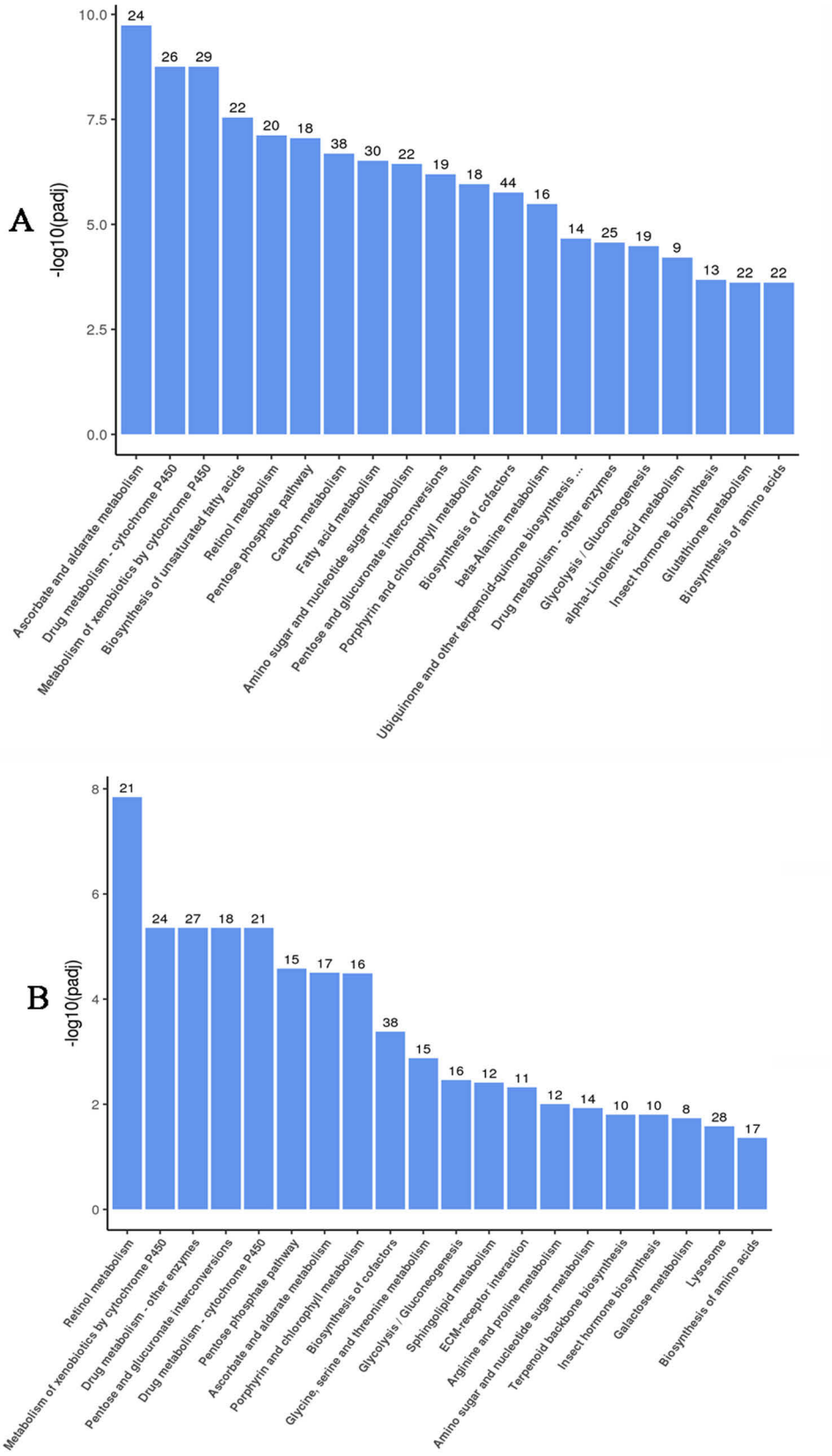

Fig. 2. The top 20 of KEGG enrichment of LH36vsCK36 and LH144vsCK144. (A) The top 20 of KEGG enrichment of LH36vsCK36; (B) The top 20 of KEGG enrichment of LH144vsCK144.

Expression patterns of factors related to glycometabolism in *S. frugiperda* larvae exposed to *B. bassiana*

In this study, the expression patterns of three signal factors associated with glycometabolism were detected after exposure to *B. bassiana*, the expression of related factors of glycometabolism was changed (Fig. 5). The relative expressions of *Sfrupdh* and *Sfrumdh* showed a similar varia-

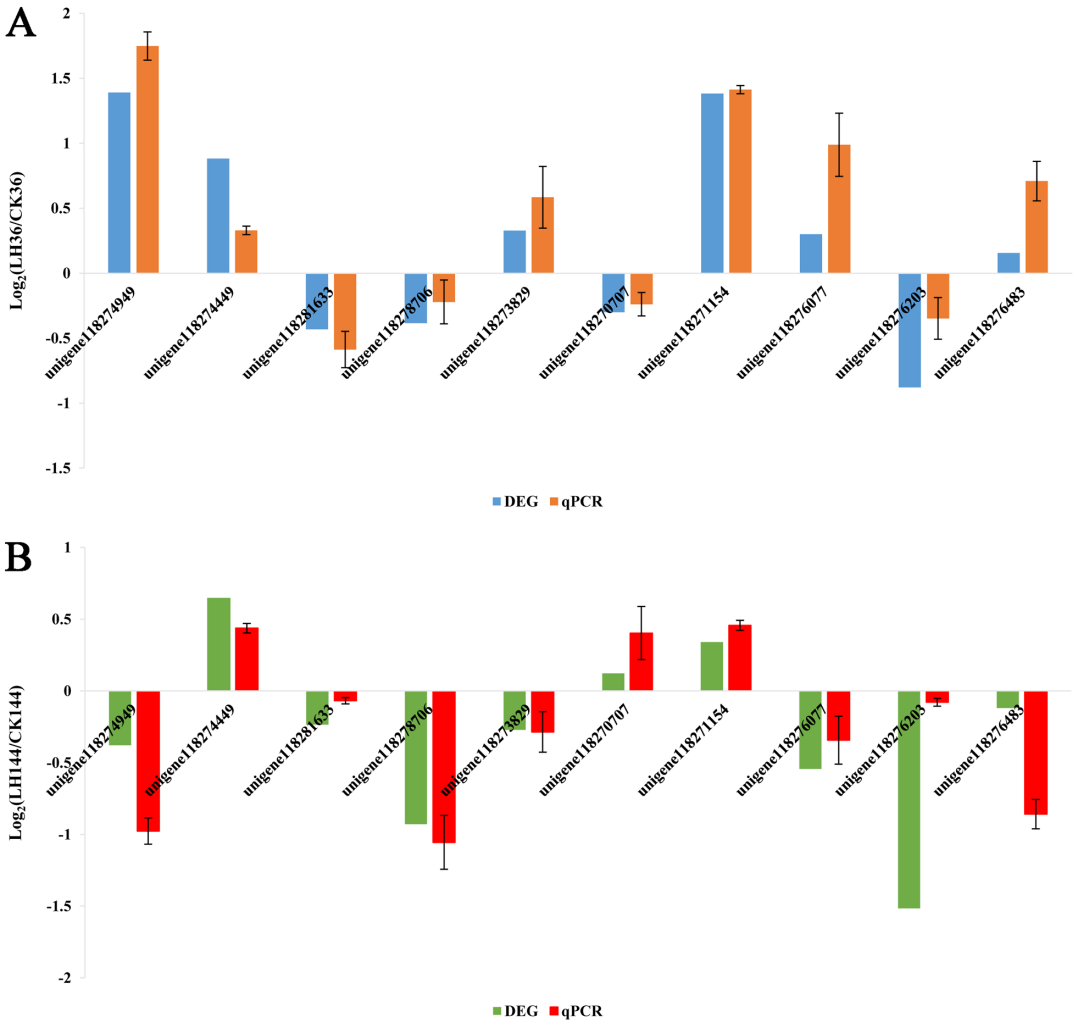

Fig. 3. Expression ratios of 10 genes in LH36vsCK36 and LH144vsCK144. (A) LH36vsCK36; (B) LH144vsCK144. Note: The fold changes of the genes were calculated as the $\log_2$ value of each comparison and are shown on the x-axis. Each error bar indicates the standard error with SEMs from the analysis of three replicates ($P < 0.05$).

tion trend with firstly up-regulation and then down-regulation, and their peaks all occurred at 36 h after exposure to *B. bassiana* (Fig. 5); the relative expression of *Sfruldh* was slightly down-regulated in the early stage of exposure, then up-regulated 48 h after exposure, and from then on, it showed a down-regulation trend (Fig. 5); the relative expression of *Sfruidh* reached the peak at 24 h after infection with *B. bassiana* and then gradually down-regulated (Fig. 5); while the relative expression of *Sfrusdh* varied with slight fluctuations from 12 h to 36 h and then sharply up-regulated 60 h after exposure (Fig. 5).

Effect of *B. bassiana* on antioxidation in *S. frugiperda*

Insects coordinate and maintain the normal physiological function of the body through antioxidation. After exposure to *B. bassiana*, the activity of GSH-PX and SOD of *S. frugiperda* (LH) was significantly increased in the early stage of exposure compared with that of unexposed *S. frugiperda* (CK), when exposed at 72 h and 48 h, GSH-PX and SOD activity were sharply decreased and significantly weaker than that of CK, respectively. The exposed *S. frugiperda* had significantly higher CAT activity at 12 h and 36 h than that of uninfected, while in other exposure stages, the exposed *S. frugiperda* showed significantly lower CAT activity compared with unexposed *S. frugiperda*. In the middle and late stages of exposure from 48 h to 72 h, GST activity was gradually becoming significantly stronger than that of CK, while in other exposure stages, the level of GST activity was lower than that of CK (Fig. 6).

Effect of *B. bassiana* on glycometabolism in *S. frugiperda*

After exposure to *B. bassiana* in *S. frugiperda*, the activities of related enzymes were varied. The activity of IDH was sharply increased from 48 h to 60 h after exposure and significantly stronger than that of CK, in other exposure stages, IDH activity was similar to that of unexposed; LDH activity was enhanced obviously in the early stage of exposure from 12 h to 36 h and then decreased compared with that of CK; the activity of MDH and PDH after infection showed a similar variation trend with that of unexposed; SDH activity showed a fluctuating changes and had a relative high level at 24 h, 36 h, and 60 h after exposure, while that of CK presented a gradually decreasing trend (Fig. 6).

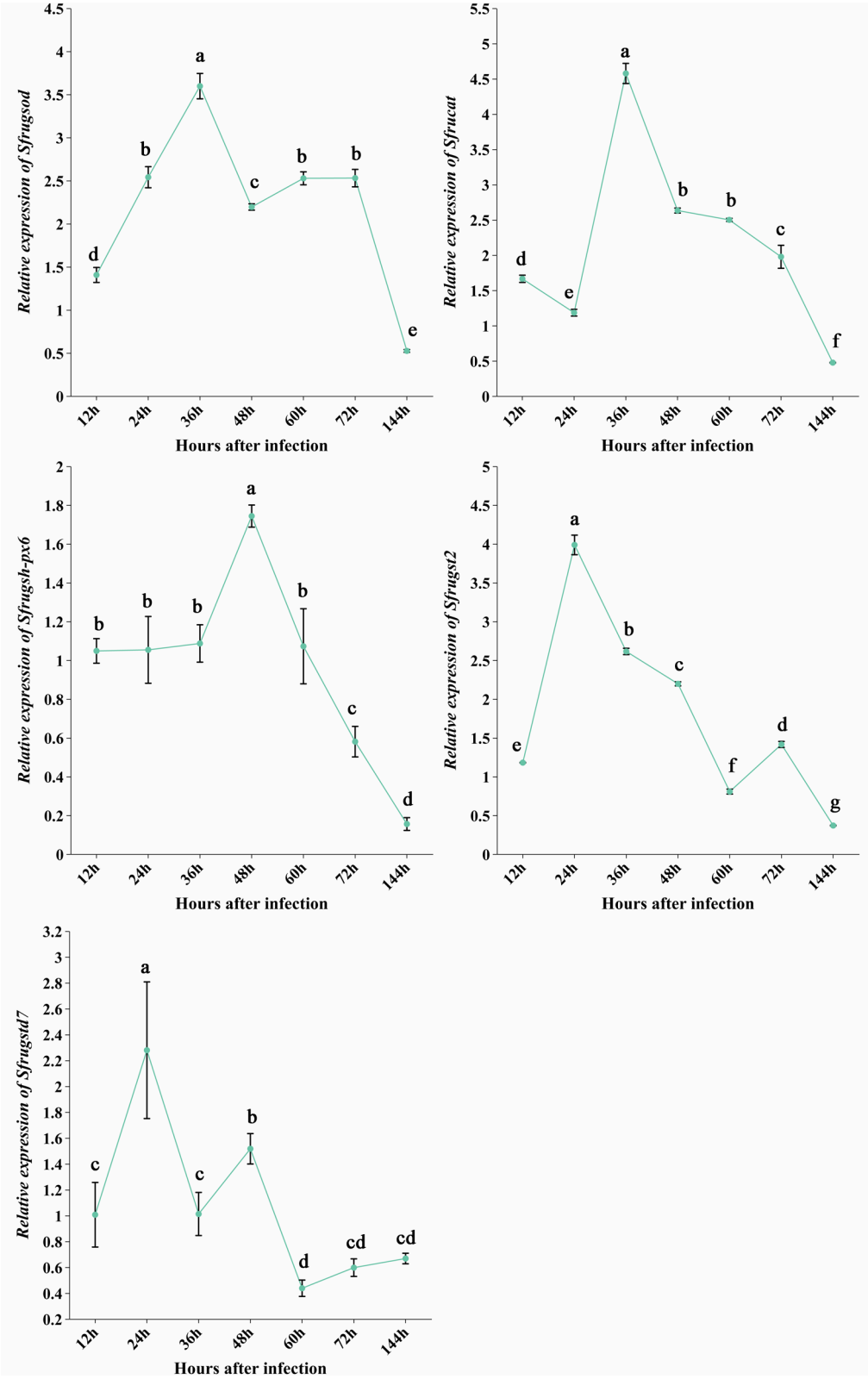

Fig. 4. Expression patterns of genes related to antioxidation in *S. frugiperda* challenged by *B. bassiana*. Relative expression levels of *sod*, *cat*, *gsh-px6*, *gst2* and *gstd7* from 12 h to 144 h in *S. frugiperda* challenged by *B. bassiana* by qRT-PCR analysis. Error bars indicate SEMs from the analysis of three replications ($P < 0.05$).

DISCUSSION

In the study, both transcriptome analyses, of *S. frugiperda* exposed to *B. bassiana* and unexposed *S. frugiperda,* were conducted using Illumina HiSeq™ 4000 technology. We obtained more than 160 GB of clean data, and DEGs in LH36vsCK36 and LH144vsCK144 libraries were identified.

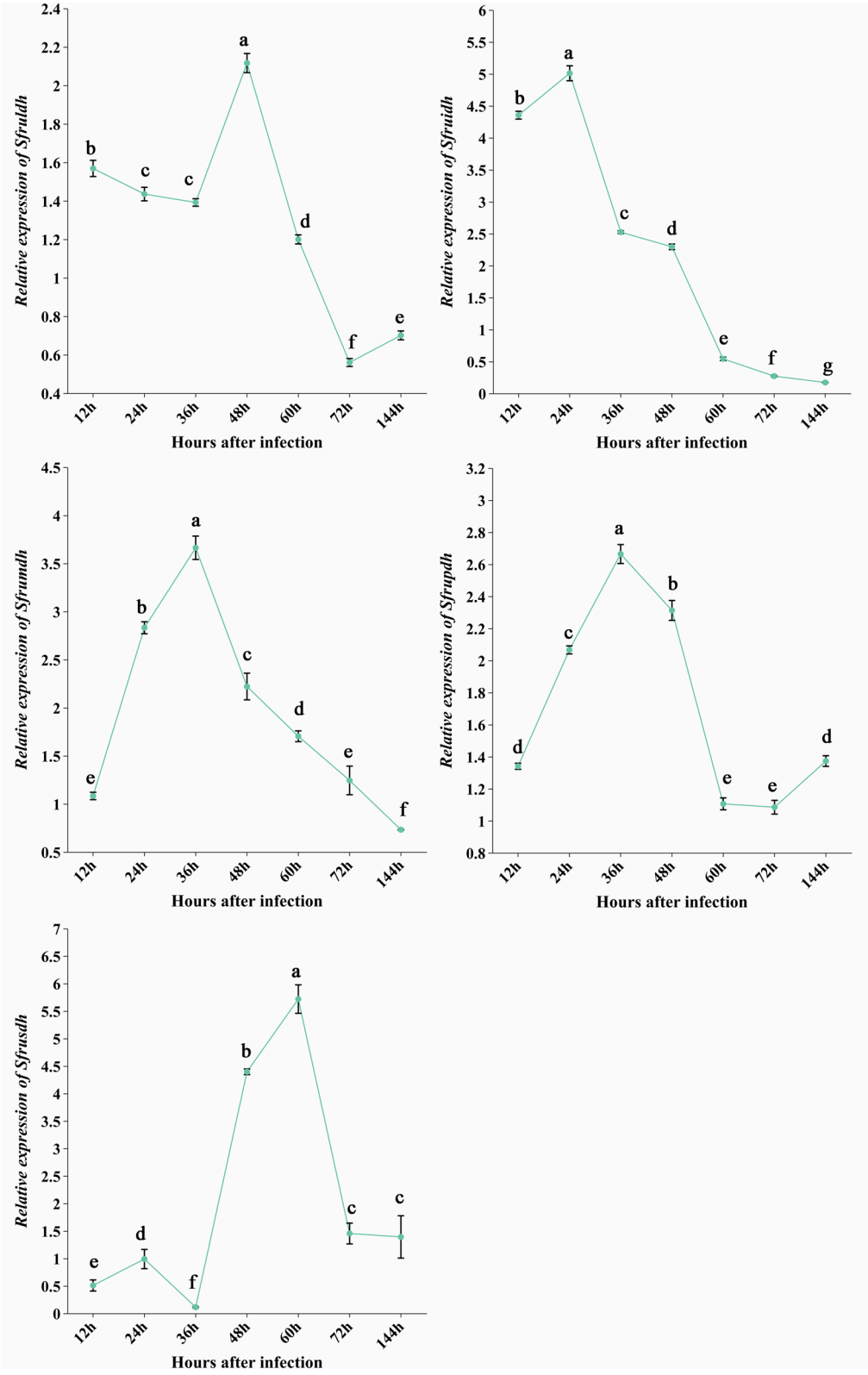

Fig. 5. Expression patterns of genes related to glycometabolism in *S. frugiperda* challenged by *B. bassiana*. Relative expression levels of *ldh*, *idh*, *sdh*, *pdh* and *mdh* from 12 h to 144 h in *S. frugiperda* challenged by *B. bassiana* by qRT-PCR analysis. Error bars indicate SEMs from the analysis of three replications ($P < 0.05$).

Studies have shown that the pathogenic mechanism of fungi is to enter the body of insects, collapse the host's defence system with rapid proliferation, and eventually lead to the death of the host due to the depletion of nutrients (Zhao, 1981). However, some recent studies have revealed that mycotoxins and the destruction of redox balance are the main causes of insect death (Ortiz-Urquiza et al., 2010). *B. bassiana* can produce a variety of toxins, including beauvericin, coccidioidin and other high molecular toxic substances (Carla et al., 2019). It has been well

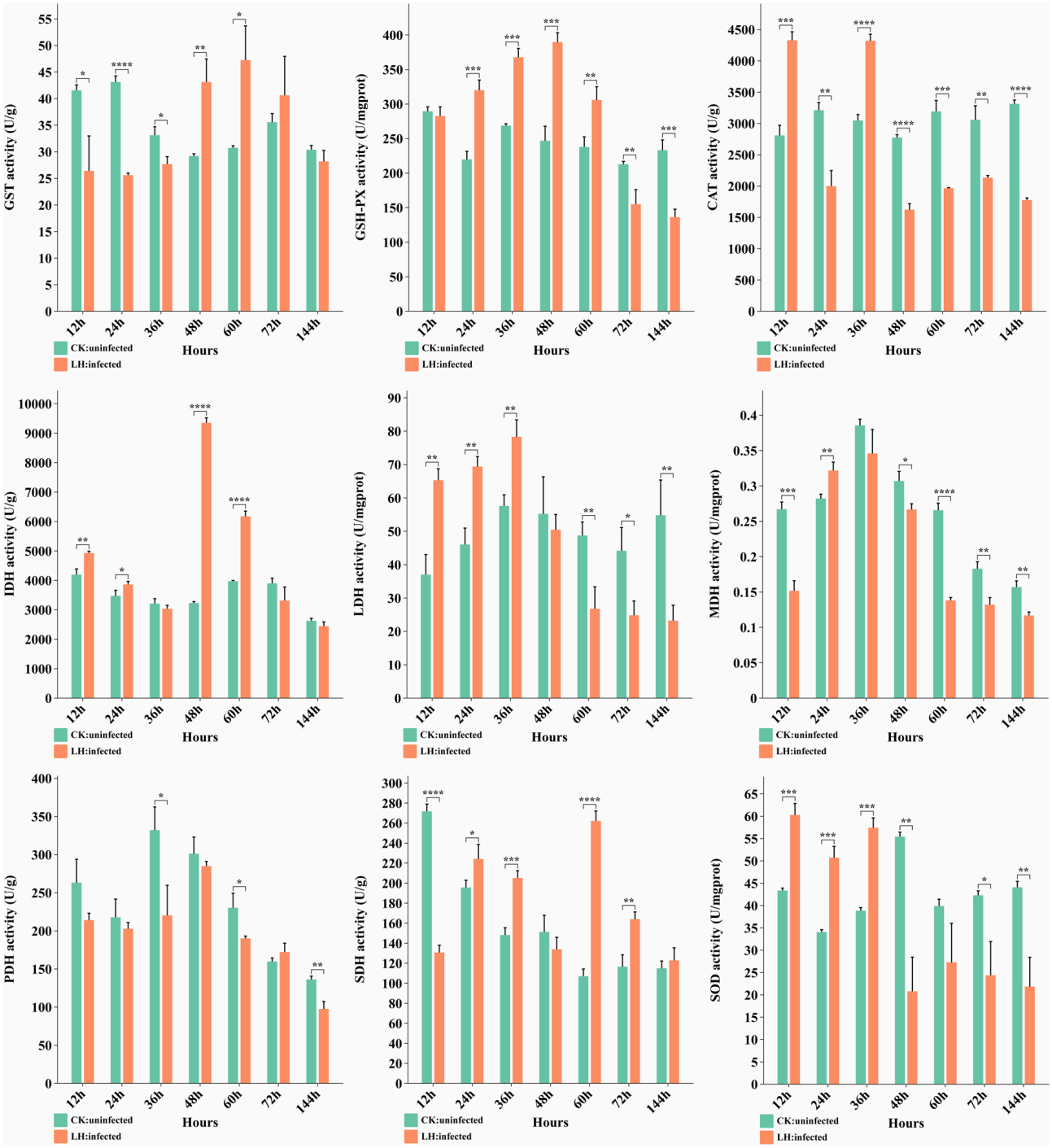

Fig. 6. Activity of enzymes associated with antioxidation and glycometabolism in *S. frugiperda* challenged by *B. bassiana*. Activity of LDH, IDH, SDH, PDH, MDH, SOD, CAT, GSH-PX6, GST2 and GSTD7 from 12 h to 144 h in *S. frugiperda* challenged by *B. bassiana* were tested. Error bars indicate SEMs from the analysis of three replications ($P < 0.05$).

established that the insecticidal effect of *B. bassiana* on the larvae of *Anopheles sinensis* and *Eurygaster integriceps*, among others, is a result of the effects of toxins (Xu et al., 2005). Real-time quantitative PCR was used to detect changes in the expression of glycometabolic enzymes and antioxidant enzymes of *S. frugiperda* after exposure to *B. bassiana*.

The results showed that the relative expressions of *Sfrupdh*, *Sfruldh*, *Sfrumdh*, *Sfrusdh*, *Sfrupdh* and *Sfruidh* were initially significantly up-regulated and then down-regulated after exposure to *B. bassiana*, suggesting that when exposed to *B. bassiana*, the larvae of *S. frugiperda* instinctively regulate the high expression of these genes in order to produce more related enzymes to maintain normal glucose metabolism. However, in the later stages of exposure, the down-regulation of glycometabolism-related genes and the significant reduction in enzyme activity in *S. frugiperda* larvae disturb glucose metabolism, which may be one of the causes leading to the death of the host (Duncan et al., 2017; Hu et al., 2021).

Toxic substances, such as mycotoxins, reactive oxygen species, pesticides, heavy metals, microplastics and other non-nutritive heterologous compounds, can have toxic effects on insects, while insects have developed a set of effective heterologous detoxification metabolic mechanisms to eliminate those threats (Yu, 2004; Wu et al., 2024). The qPCR results revealed that the relative expression levels of *Sfrusod* and *Sfrucat* were significantly upregulated during the initial phase of *B. bassiana* exposure. This early response suggests an activation of the host's defense mechanisms. Furthermore, the assessment of antioxidant activity demonstrated a modest increase in both superoxide dismutase (SOD) and catalase (CAT) activities at early and middle stages of infection. This slight elevation in enzymatic activity implies an initial surge in free radical production following *B. bassiana* invasion, indicative of the host's attempt to counteract oxidative stress. However, as the infection progressed in later stages, a marked decrease in the relative expression of the aforementioned genes plus a significant reduction in antioxidant activity were observed. This decline strongly suggests that the antioxidant defenses of *S. frugiperda* was severely compromised, potentially leading to an overwhelming oxidative burden that the host could not manage effectively (Zhang et al., 2023). Drawing from these findings, *B. bassiana* disrupts the delicate antioxidant balance within *S. frugiperda*, contributing significantly to the pathogen's lethal effects. The disruption of this equilibrium likely exacerbates oxidative damage, overwhelming the host's cellular repair mechanisms and culminating in the demise of the insect (Zhang et al., 2023). *B. bassiana*'s pathogenic strategy involves the secretion of toxins that not only perturb the host's normal metabolic processes, but also dismantle the intricate antioxidant defense system of *S. frugiperda*. This dual assault on the insect's physiology is a critical factor in the pathogen's virulence, ultimately leading to the host's death. Our study underscores the importance of understanding the complex interplay between pathogen-derived toxins and host antioxidant responses in the context of insect-pathogen interactions.

GSTs and GSH-Px are important detoxification and antioxidant enzymes, which not only participate in the detoxification process of exogenous heterologous toxic substances such as insecticides, heavy metals, fluoride and other substances, but also participate in the removal of endogenous toxic substances such as reactive oxygen species produced in the metabolic process of life (Kolawole et al., 2014; Kshatriya & Gershenzon, 2024). It is one of the most important detoxification and antioxidant defence system in insects.

During the intermediate and advanced phases of *B. bassiana* infection, the relative expression levels of *Sfrugst2* and *Sfrugsh-px6* were observed to be upregulated to varying degrees. This upregulation, coupled with an enhanced enzymatic activity of GSTs and GSH-PX, suggests that the *Sfrugst* and *Sfrugsh-px* genes may serve as principal detoxification genes in *S. frugiperda* larvae. These genes are instrumental in combating the toxins introduced by *B. bassiana*, highlighting their crucial role in the host's defensive mechanisms against fungal invasions (Strange et al., 2001; Han et al., 2013). As the infection progresses, the proliferation of *B. bassiana* spores and mycelium leads to increased toxin production. This triggers an increase in the enzymatic activity of GSTs and GSH-PX, which are integral to the detoxification and antioxidant processes, safeguarding the larval cells from oxidative damage. Concurrently, there is a substantial consumption of GSH, leading to a critical depletion that the larvae's system cannot replenish sufficiently to meet the heightened demand. This GSH deficit culminates in the death of the larvae during the late stages of infection, underscoring the significance of an adequate detoxification and antioxidant response (Liu et al., 2015; Gullner et al., 2018). The exposed larvae of *S. frugiperda*, in an effort to bolster their defenses, appear to modulate the expression of these detoxification and antioxidant genes in an effort to amplify their detoxification and antioxidant capabilities. However, in the terminal stages of infection, *B. bassiana* disrupts the insect's redox balance – a delicate equilibrium of oxidative and reductive processes. The secondary metabolites secreted by *B. bassiana* exert a detrimental impact on the cellular and tissue integrity of the insect, culminating in metabolic disarray. This metabolic turmoil ultimately leads to the demise of *S. frugiperda*, illustrating the complex interplay between pathogen virulence and host resistance (Wang et al., 2021; Aioub et al., 2023; Sun et al., 2023).

This study highlights the pivotal role of detoxification and antioxidant defense mechanisms in *S. frugiperda* larvae, particularly in countering fungal infections at physiological, biochemical, and transcriptional levels. It has underscored the importance of these processes in the host's defense against mycotoxins, highlighting the need for further exploration into the regulatory mechanisms of *S. frugiperda*'s detoxification and antioxidant systems, as well as the antifungal and pathogenic mechanisms of the fungi themselves. This research not only deepens our understanding of host-fungus interactions but also lays a foundational scientific groundwork for the development of novel fungal agents and the management of detrimental agricultural and forestry pests. In parallel, the ecological role of *B. bassiana* and its interplay with host responses to infection, alongside the influence of pesticides and herbicides on these dynamics, is critical for the strategic deployment of biocontrol in agricultural ecosystems. Current research indicates that the impact of herbicides on *B. bassiana* is intricate and varies with the type, concentration, and application method of the herbicide in question (Celar & Kos, 2016; Vommaro et al., 2023). In the context of agricultural practices, it is imperative to apply herbicides with an awareness of their potential effects on biological control agents. Where feasible, there should be a preference for alternatives that exert minimal impact on beneficial microorganisms like *B. bassiana*.

By integrating these insights, we could foster a more effective and sustainable approach to pest management. This involves leveraging the natural defenses of pests like *S. frugiperda* and the biocontrol potential of fungi such as *B.*

bassiana, while being mindful of the environmental implications of chemical use. Such an integrated strategy will not only enhance the efficacy of biological control agents but also minimize the reliance on chemical pesticides, thereby reducing environmental risks and advancing the cause of sustainable agriculture.

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Table S1. Primers of the candidate glycometabolism and antioxidation-related enzyme genes used in qRT-PCR.

Gene	Primer sequences (5'–3')	S/AS
gadph	ACCAACTGTCTCGCTCCTCT	S
	GAGGGTCCGTCAACAGTCTT	AS
ldh	CCCAATGTGGACAATGACG	S
	CGGAGTGCAATGTTACGG	AS
idh	GTTCCGCTATCTGCTCT	S
	GTCCAAGCCAATGTTCT	AS
mdh	CCGTCATCGCCGCAAGAAAG	S
	AACCATCGGAGACAACACCC	AS
pdh	CCACAACTGCTACCCTAACCA	S
	GAAACCACGGATGATCTTCTC	AS
sdh	AGGAGGTGGTGGACGGAGTA	S
	GCAGTAAGGCTTGCGACA	AS
cat	AGATGCTTCTTTGTTCCCTA	S
	CCGTTCATGTGCCTGTAA	AS
gsh-px6	ATGCTCCTGTTAGGCGATTT	S
	ACTTTGGTATTGCGTTTCC	AS
gst2	CCCAGACCTATTGGAGAAA	S
	AGTCAAACATACCAGCGAAC	AS
gstd7	CGGAGTTTGTCGTTTGTGG	S
	CGGAGAAGGTGGGAGGTAG	AS
sod	CCCCAGCAAGATGGAGCA	S
	TGACCACCAAGGCCGAAG	AS

Table S2. Primers used in qRT-PCR.

Gene ID	Primer sequences (5'–3')	S/AS
118271154	CCACAACTGCTACCCTAACCA	S
	GAAACCACGGATGATCTTCTC	AS
118274949	GGACGATAGAGGTTGAAGAA	S
	TGGAAAGTTGTAGGGATGG	AS
118274449	CAGAGCAACGACAACAG	S
	ATAGGTCAGCACGAGCA	AS
118281633	GATGTGTGGGCTTATGGGG	S
	ACCTGGGTGTGTTGTCTGG	AS
118278706	TCCTCCGAGACTCTGCC	S
	CGGGGTCCTTGTAATGC	AS
118273829	TATTCTCTGTGTGTCCTTCCG	S
	ACCTATTTAGTGGCCTTGCTA	AS
118270707	GAGACACAGGTATCCCAAA	S
	TCAGCACAGCATTATCATC	AS
118276077	GTTTACAGATTTACCGTCCCT	S
	TATTCGTTAGTTGGTTAGCAT	AS
118276203	TCTGGTTTGATTACGCTTTT	S
	TGGAGTTCATTTTGGATTTT	AS
118276483	CAAAGTAGTCTCGCTCCG	S
	TTTCTTAATTCCCGCAGT	AS

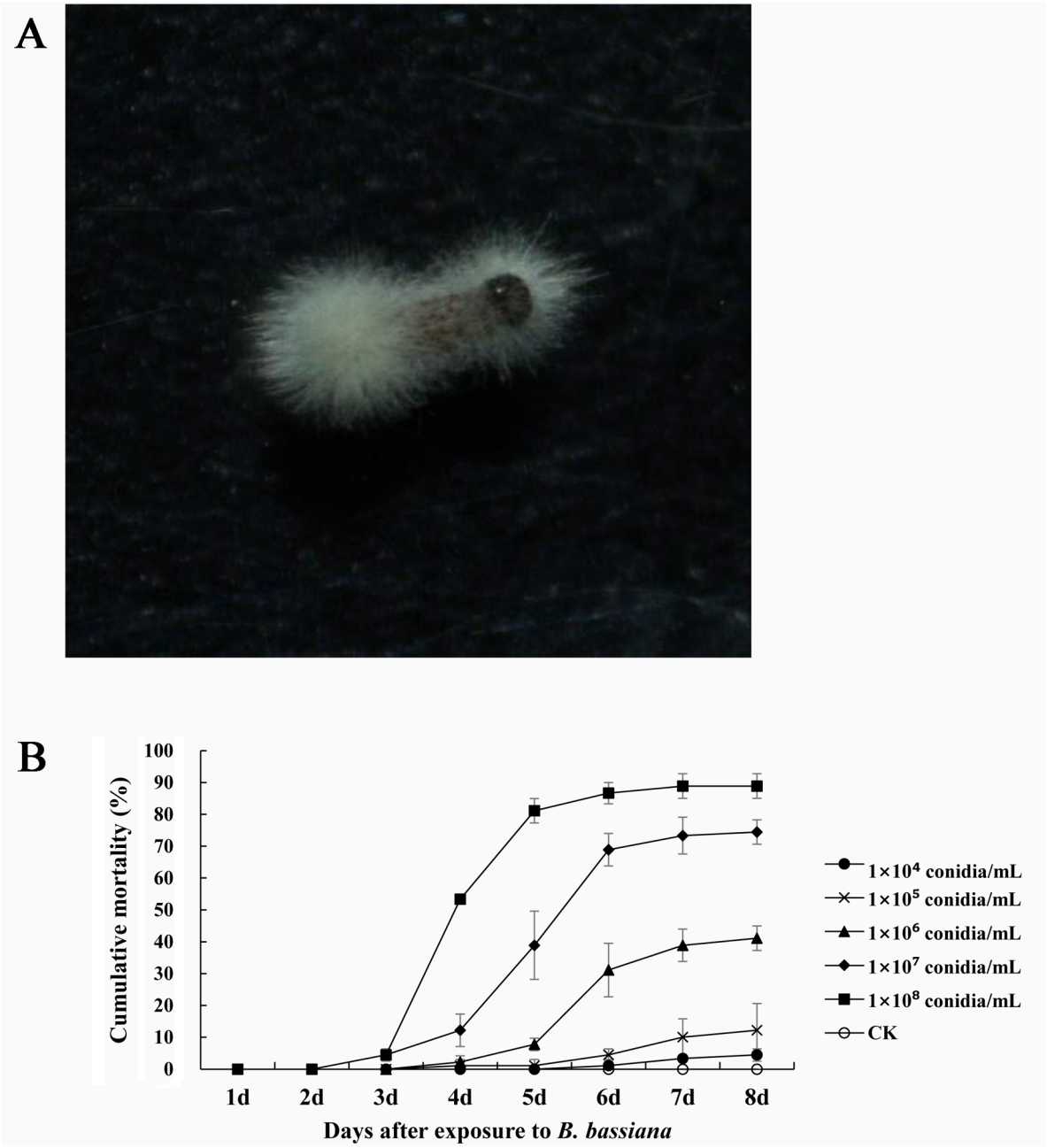

Fig. S1. (A) The *S. frugiperda* larvae were checked after death to confirm mycelium growth. (B) Cumulative mortality of third instar larvae of *S. frugiperda* exposed to *B. bassiana* spore suspension with different concentrations. Note: CK – without exposure to *B. bassiana*. The data in the figure are mean ± standard deviation.