Cloning and Functional Characterization of a Novel $\beta$-GRP Gene From Melanotus cribricollis

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Abstract

In this study, a novel $\beta$-1,3-glucan recognition protein gene ($\beta$-GRP) was identified from Melanotus cribricollis, and its potential role in the immune response was investigated. The full length of $\beta$-GRP cDNA (Accession Number: MT941530) was 1644 bp, encoding a protein composed of 428 amino acids. The theoretical molecular weight and the isoelectric point were 51.53 kDa and 6.17, respectively. The amino acid sequence of $\beta$-GRP from M. cribricollis was closely related to that of $\beta$-GRP-like from Photinus pyralis, and was predicted to contain conserved GH16 domain without glucanase active site. The results of real-time quantitative PCR showed that fungal infection of Metarhizium pingshaense may significantly upregulated the expression level of $\beta$-GRP gene. The RNAi suppression of $\beta$-GRP gene expression significantly increased the corrected cumulative mortality. Meanwhile, antimicrobial peptide genes defensin and lysozyme were significantly downregulated after interference of $\beta$-GRP. Taken together, these results suggest that $\beta$-GRP of M. cribricollis probably participates in the host immune system by mediating the expression of antimicrobial peptides. This study provides comprehensive insights into the innate immune system of insect larvae.

Graphical Abstract

The innate immune system is the basic defense system of insects against pathogen invasion because they lack adaptive immunity (Ochiai and Ashida 2000). In response to pathogen attack, the pattern recognition receptors (PRRs) of insects bind with pathogen-associated molecular patterns (PAMPs) and then activate downstream pathways, mainly involves phenoloxidase (proPO)
cascades, Toll and Imd signaling pathway, which further promote the melanization cascade or induce the production of antimicrobial peptides (AMPs) (Hoffmann 2003, Mishima et al. 2009, Matskevich et al. 2010). Insect PRRs include proteins, such as β-1,3-glucan recognition protein (β-1,3-GRP), Gram-negative bacteria-binding protein (GNBP), and peptidoglycan recognition protein (PGRP), among which β-1,3-GRP and GNBP are classified into a subfamily of PRR because of their high homology (Royer 2004, Vogel et al. 2014). β-1,3-GRP was first thought as component of the proPO cascade in silkworm (Ochiai and Ashida 1988). A previous study reported that β-1,3-GRP/GNBP3 is a key PRR with strong affinity with β-1,3-glucan, a cell wall component of fungi. Upon binding to fungi, it evokes the Toll and proPO pathways in invertebrates to defend against fungal invasion (Takahasi et al. 2009). Insect βGRP homologs consist of a highly conserved N-terminal domain and a β-1,3-glucanase-like domain in the C-terminal region (Ochiai and Ashida 2000, Hoffmann 2003). Accordingly, β-1,3-GRP is an important component of insects to resist foreign invaders (Lemaître and Hoffmann 2007, Dai et al. 2013). To date, the function of the β-1,3-GRP homolog has been well described in different insects but remains elusive in Melanotus cribricollis (Faldermann) (Coleoptera: Elateridae) larvae.

Larvae of M. cribricollis are the dominant species of bamboo shoot worms, which are important shoot-stage pests in bamboo forests. The damage rate of bamboo forests can be as high as 80%. The number of insects feeding on a fresh bamboo shoot can reach nearly 20. M. cribricollis lives underground and has a long history, making it difficult to prevent and control (Shu et al. 2012). Previously, the control of bamboo shoot wireworms in bamboo forests relied on chemical agents, such as fipronil, chlorpyrifos, imidacloprid, and phoxim (Song et al. 2009). However, chemical agents such as chlorpyrifos have been gradually banned because of the increasingly prominent problems of pesticide residues, environmental pollution, and drug resistance. Biocontrol bacteria, such as Metarhizium pingshaense Q.T. Chen & H.L. Guo (Ascomycota: Hypocreales), have the advantages of environmental protection and sustainability, and they have been widely studied and applied in recent years. M. pingshaense has pathogenic effects on more than 200 pests, including those belonging to the orders Coleoptera, Lepidoptera, Orthoptera, Diptera, and Homoptera, and is a biocontrol strain with great potential (Roberts and St Leger 2004).

In our previous study, we identified using next-generation sequencing (RNA-seq) that β-GRP/GNBP genes are significantly upregulated after infection with M. pingshaense WP08 strain for 7 d (Ye et al. 2018). This study suggests that β-GRP of M. cribricollis was probably involved in immune regulation. The current research on insect immune mechanism focused on the silkworm and fruit flies, and few reports elucidated the immune mechanism of underground phytophagous pests. Identifying the immune-related genes of M. cribricollis in bamboo forests will provide not only reference information for immune mechanism research in other insect but also provide target genes for transgenic insect resistance technology and lay a foundation for the development of high-efficiency biocontrol strains that inhibit the insect immune system.

**Materials and Methods**

**Animals and Pathogens**

Healthy larvae of M. cribricollis with the same body weight were collected from the bamboo forest of Zaoyuan in Deqing County, Zhejiang Province, China and fed with fresh bamboo shoots indoors. M. pingshaense WP08 strain was provided by the Forest Protection Research Group of the Institute of Subtropical Forestry, Chinese Academy of Forestry. It was inoculated on potato dextrose agar and cultured at 25°C for approximately 3–4 wk to collect conidia for use. The test soil was collected from the early bamboo garden in Hangzhou, sieved through a 40-mesh screen, sterilized at high temperature (121°C) for 1 h, and then dried. A M. pingshaense WP08 conidia suspension was prepared using 0.1% Tween 80 and then mixed with the sterilized test soil for use. The final concentration of conidia was adjusted to 1.2 × 107 pcs·g⁻¹ soil. Humidity was adjusted to 10% ± 1% (TZS Soil Moisture Quick Tester, Zhejiang Tuopu Instrument Co., Ltd.).

**Cloning of Full-length β-GRP**

**Total RNA Isolation, cDNA Synthesis, and Gene Cloning**

The middle part of the M. cribricollis larval body was cut with scissors, placed in a tube, and then quickly placed in liquid nitrogen to cool down to prevent RNA degradation. Total RNA was extracted using TRIzol reagent (Takara, Japan). RNA integrity, concentration, and purity were detected using RNA electrophoresis and a NanoDrop 2000 spectrophotometer (Thermo, USA). cDNA first-strand synthesis was performed using the PrimeScript Reagent kit with gDNA Eraser (Takara, Japan) in accordance with the manufacturer’s instructions. The primer pair (Mcβ-GRP-734F/Mcβ-GRP-1152R) was designed according to the Unigene C54949.Graph_c0 sequence acquired by the transcriptomic data of M. cribricollis in our previous study (Ye et al. 2018) and used to amplify the intermediate fragment of β-GRP (Table 1). The 3' RACE cDNA and 5' RACE cDNA of the targeted β-GRP gene were synthesized using the 3' RACE kit (Biotek, China) and SMARTer RACE kit (Takara, Japan), respectively. The specific primers are shown in Table 1. The RACE-PCR program was performed following the manufacturer’s protocol. The PCR products were purified with the Gel Recovery Kit (TSINGKE, China) in accordance with the manufacturer’s protocol. Obtained β-GRP DNA fragments were further sequenced using Tsingke Biotechnology Co., Ltd. (Beijing, China).

**Bioinformatics Analysis**

The open reading frame (ORF) of β-GRP was predicted using the ORF Finder program (Stothard 2000). Physical properties, including molecular weight, theoretical isoelectric point (pI), grand average of hydropathicity (GRAVY), protein hydrophobicity (Wilkins et al. 1999), membrane structure (Krog et al. 2001), signal peptide (Nielsen et al. 2019), phosphorylation site (Blom et al. 2004), and glycosylation site (Gupta et al. 1999), were predicted. Multiple alignment and phylogenetic analysis were performed based on amino acid sequences of recognition protein from M. cribricollis (β-GRP), Photinus pyralis (Linnaeus) (Coleoptera: Lampyridae) (β-GRP-like, XP031347801.1), Agrius planipennis Fairmaire (Coleoptera: Buprestidae) (β-GRP1-like, XP025830502.1), Tenebrio molitor Linnaeus (Coleoptera: Tenebrionidae) (GNBP1, BAG142631.1; β-GRP AC99308.1), Tribolium castaneum (Herbst) (Coleoptera: Tenebrionidae) (GNBP2, NP01164284.1), Tribolium madens (Charpentier) (Coleoptera, Tenebrionidae) (β-GRP-like XP044269569.1), Anopheles gambiae sensu stricto (Diptera:Culicidae) (GNBP-B1, ABU80032.1), Nasutitermes cornutus (Isoptera, Nasutitumidae) (GNBP1, AAZ84864.1), and four glucanases sequences from Periplaneta americana (Linnaeus) (Blattodea: Blattidae) (ABR28480.1), Nocardiopsis sp (Streptosporangiales: Nocardiopsaceae) (BAE54308.1), T. molitor (FJ864682), Spodoptera frugiperda (JE Smith) (Lepidoptera : Noctuidae) (ABR28478.2). The
of 0.1% Tween 80 (control group). The setting of conidia concentration and soil humidity was described above. Five individuals of M. cribricollis larvae were randomly sampled in the experimental groups at four time periods (day 0, 7, 12, and 17). Three replicates were set up for each treatment. The tissue was further used for total RNA isolation and subsequent qPCR experiments.

In the immune stimulation experiment, healthy M. cribricollis larvae were divided into two groups. In one group, M. cribricollis were raised in soil treated with equal volume of 0.1% Tween 80 (control group). The setting of conidia concentration and soil humidity was described above. Five individuals of M. cribricollis larvae were randomly sampled in the experimental and control groups at four time periods (day 0, 7, 12, and 17). Three replicates were set up for each treatment. The tissue was further used for total RNA isolation and subsequent qPCR experiments.

The template cDNA was synthesized for quantitative real-time PCR (qRT-PCR), and the primer pairs for the targeted genes are shown in Table 1. dsRNA synthesis was performed using the T7 RiboMAX Express kit (Promega, USA) in accordance with the manufacturer’s instructions. RNA interference was completed via intramuscular injection with 500 ng of dsRNA dissolved in 20 μL of PBS buffer through the connection of the 3rd and 4th abdominal segments of M. cribricollis larvae. Larvae of the experimental group were injected with β-GRP dsRNA, and the control groups were injected with the same amount of PBS buffer or dsGFP. At 48 h after injection, five larvae were collected from each group, and the interference effect was detected using qRT-PCR. Larvae treated with PBS group were assumed as control. Three replicates were set up for each sample. RPS27a and RPS3 gene was both selected as two housekeeping genes for internal normalization as described in our previous study (Ye et al. 2021), and averaged expression values of targeted genes relative to two housekeeping genes were taken for subsequent analysis. The gene expression level of larvae from control group at day 0 was assumed arbitrarily as 1. The relative mRNA expression level was calculated using the 2−ΔΔCt method (Livak and Schmittgen 2001). Analysis of variance was performed using SPSS software. Statistical significance was analyzed by Student’s t-test.

### Expression Change in β-GRP under Infection with M. pingshaense

In the immune stimulation experiment, healthy M. cribricollis larvae were divided into two groups. In one group, M. cribricollis were raised in soil treated with M. pingshaense. In the other group, M. cribricollis were cultured in sterilized soil treated with equal volume of 0.1% Tween 80 (control group). The setting of conidia concentration and soil humidity was described above. Five individuals of M. cribricollis larvae were randomly sampled in the experimental and control groups at four time periods (day 0, 7, 12, and 17). Three replicates were set up for each treatment. The tissue was further used for total RNA isolation and subsequent qPCR experiments.

The template cDNA was synthesized for quantitative real-time PCR (qRT-PCR), and the primer pairs for the targeted genes are shown in Table 1. dsRNA synthesis was performed using a cDNA template containing the 2−ΔΔCt method (Livak and Schmittgen 2001). Analysis of variance was performed using SPSS software. Statistical significance was analyzed by Student’s t-test.

### RNA Interference by Double-strand RNA Injection

The double-strand RNA (dsRNA) of β-GRP and green fluorescent protein (GFP) were synthesized using a cDNA template containing the T7 polymerase promoter, and the specific primers are shown in Table 1. dsRNA synthesis was performed using the T7 RiboMAX Express kit (Promega, USA) in accordance with the manufacturer’s instructions. RNA interference was completed via intramuscular injection with 500 ng of dsRNA dissolved in 20 μL of PBS buffer through the connection of the 3rd and 4th abdominal segments of M. cribricollis larvae. Larvae of the experimental group were injected with β-GRP dsRNA, and the control groups were injected with the same amount of PBS buffer or dsGFP. At 48 h after injection, five larvae were collected from each group, and the interference effect was detected using qRT-PCR. Larvae treated with PBS group were assumed as control. Three replicates were set up for each sample. RPS27a and RPS3 gene was both selected as two housekeeping genes for internal normalization as described in our previous study (Ye et al. 2021), and averaged expression values of targeted genes relative to two housekeeping genes were taken for subsequent analysis.

### Table 1. Summary of primers used in this study

| Primers | Purpose | Sequences (5′–3′) |
|---------|---------|------------------|
| Mcβ-GRP-734F | PCR of intermediate fragment | AATCAGCCAGAATAAGTCAC |
| Mcβ-GRP-1152R | PCR of intermediate fragment | CCTCAGGAAAGTCACAGGAA |
| Mcβ-GRP-3′ RACE Inner1 | 2nd PCR of β-GRP | AAGCAGACAGTGAAGAT |
| 5′ GSP | 1st PCR of β-GRP | GATTACGCCAACGTTCGAGGTCT |
| 5′ NGSP | 2nd PCR of β-GRP | GATTACGCCAACGTCGGGACCAT |
| Mcβ-GRP-qF | qRT-PCR of β-GRP | CCCCCAGACATTTCTCTGTATTG |
| Mcβ-GRP-qR | qRT-PCR of defensin | ATCCATTTGAGTTAGTGGG |
| Def c44149.graph_c0/F1 | qRT-PCR of lysozyme | TGAGAAAGCAGTAGAAGTG |
| Def c44149.graph_c0/R1 | qRT-PCR of PGRPs | GTATTGACGAAAATCTTTGGG |
| Lys c48579_graph_c0/F | qRT-PCR of PGRPs | CGTGTTGCACAAATCTGAACCT |
| Lys c48579_graph_c0/R | qRT-PCR of PGRPs | TCGTCGATCTCTGCTATCTAG |
| PGRP c22434.graph_c0/F1 | qRT-PCR of PGRPs | TGGATGAGGGGGTTTACTTGC |
| PGRP c22434.graph_c0/R1 | qRT-PCR of PGRPs | CTGGTCGGTAATCTTTGCTTG |
| RPS27a-qF | Reference gene | GCTTTTTGGTAGCCTGTCAT |
| RPS27a-qR | Reference gene | GATATAGGAGAAAACAGCAC |
| RPS3-qF | Reference gene | TGTATTTGAGGAGGAG |
| RPS3-qR | Reference gene | CAAATGCGACCAAAAACAGC |
| GFP-F | dsRNA synthesis of GFP | ATGTTGACGACAAGGCGAGGAG |
| GFP-R | dsRNA synthesis of β-GRP | TTTACTTGTAGCTGGTCCATGG |
| T7-GFP-F | dsRNA synthesis of β-GRP | gatccacattagttctagcatagttatg |
| T7-GFP-R | dsRNA synthesis of β-GRP | CTGGAGCTTTCCGGTTATAT |
| Mcβ-GRP-F | dsRNA synthesis of β-GRP | TGGTTGTAGAATATCTCATG |
| Mcβ-GRP-R | dsRNA synthesis of β-GRP | gatccacattagttctagcatagttatg |
| T7-Mcβ-GRP-F | dsRNA synthesis of β-GRP | TTATTTTGGACTGGAGAG |
| T7-Mcβ-GRP-R | dsRNA synthesis of β-GRP | gatccacattagttctagcatagttatg |

Nucleotides with lowercase represent the T7 promoter sequence for double-strand RNA synthesis.
each treatment. Statistical analysis was performed using SPSS 19.0 software. Significant differences were identified by Student’s t-test. Analysis of variance was performed using SPSS software.

**Immune Functional Characterization of β-GRP**

After injecting β-GRP-specific dsRNA or GFP-specific dsRNA or PBS for 48 h, we isolated total RNA from the middle part of the body of *M. cribricollis* and reverse transcribed it to cDNA. The relative expression levels of two AMP genes *defensin* and *lysozyme*, and one *PGRP* gene were analyzed using qRT-PCR, and the primer pairs for the targeted genes are shown in Table 1. Five larvae were randomly sampled in each group with three replicates. Larvae treated with PBS group were assumed as control.

Larvae of three treatment groups injected with PBS, GFP, and β-GRP for 48 h, respectively, then raised in soil treated with *M. pinghaense*. Larvae of three corresponding control groups were raised in soil treated with equal volume of 0.1% Tween 80. The setting of conidia concentration and soil humidity was described above. Each group contained 20 larvae. The corrected cumulative mortality was calculated according to the following formula:

\[
\text{Corrected cumulative mortality} = \frac{\% \text{ test mortality} - \% \text{ control mortality}}{100 - \% \text{ control mortality}} \times 100
\]

Analysis of variance was performed using SPSS software. Statistical significance was analyzed by Student’s t-test.

**Results**

**Molecular Cloning and Bioinformatics Analysis of β-GRP**

The cloned sequence of β-GRP cDNA was submitted to the GenBank database (accession NO. MT941530). The full-length was 1644 bp with a complete ORF of 1395 bp encoding a polypeptide consisted of 464 amino acids. The ORF sequence was selected from 38 bp to 1432 bp with starting codon ATG and ending codon TAG. The lengths of 5’ UTR and 3’ UTR were 37 and 212 bp, respectively. The putative molecular weight of β-GRP protein was 51.53 kDa, and the pl value was 6.17.

Signal peptide was analyzed. A signal peptide composed of 26 amino acids was found in the N-terminal, and the cleavage site was between positions 26 and 27 (LYA-IP). DictyOGlyc1.1 glycosylation sites located outside the membrane. The protein sequences, no catalytic region was found in other seven recognition protein sequences from *M. cribricollis* β-GRP-like, *A. planipennis* GRP1-β, *T. molitor*, and *T. castaneum*.

**Sequence comparison between GH16 domain of several recognition protein sequences:**

Sequence comparisons between GH16 domain of several recognition protein sequences from *M. cribricollis* β-GRP-like, *A. planipennis* GRP1-β, *T. molitor*, and *T. castaneum*. Four glucanases sequences from *N. comatus* (FP031348780.1), *A. planipennis* (AC99308.1), *T. molitor* (NP001164284.1), and *T. castaneum* (XP044269659.1) were clustered with *M. cribricollis* β-GRP protein sequences from *clustered with M. cribricollis β-GRP, AC99308.1, T. molitor, NP001164284.1, T. castaneum*.

**Fig. 1. Sequence of β-GRP cDNA nucleotide and translated amino acid.**

CBM39 and GH16 binding domain covering residues 27–132 and 152–463, respectively. The β-GRP protein was inferred to be hydrophilic based on their few hydrophobic amino acids and GRAVY value of −0.252.

The results of BlastP showed that the β-GRP amino acid sequence contains the β-1,3-glucan recognition protein conserved domain of the insect GRP glycoside hydrolase 16 superfamily (GH16 β-GRP, LamG superfamily) at the C-term. The lengths of 5’ UTR and 3’ UTR were 37 and 212 bp, respectively (Fig. 1). The putative molecular weight of β-GRP protein was inferred to be hydrophilic based on their few hydrophobic amino acids and GRAVY value of −0.252.

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glucanases sequences cluster into the clade 2 with other two recognition protein sequences from *A. gambiae* and *N. comatus* (Fig. 2B).

**Expression of β-GRP Stimulates with *M. pingshaense***

We analyzed the time-course expression profile of β-GRP to test whether β-GRP is involved in the innate immune response of *M. cribricollis* larvae. On day 7, β-GRP expression significantly upregulated (fold change >5) in the experimental group compared with the control group and remained significantly higher (fold change >5) until day 17 (Fig. 3). This result indicates that β-GRP probably participated in regulating the immune system of *M. cribricollis* larvae to resist the invasion of pathogenic microorganisms.

**Double Strand RNA Interference***

We developed dsRNA interference to confirm the immune function of β-GRP in *M. cribricollis* larvae. About 500 bp β-GRP gene-specific target for dsRNA synthesis were selected. For the negative control group, we synthesized GFP gene-specific dsRNA, whose gene does not exist in *M. cribricollis* larvae. As shown in Fig. 4, the expression level of β-GRP in the β-GRP interference group was significantly downregulated (fold change >6.5) compared with that in the GFP silenced and PBS groups. This result suggested that β-GRP mRNA was successfully silenced in vivo, and the corresponding dsRNA can be used for further experiment.

**Knockdown of β-GRP Gene Decreased the Survival Rate with *M. pingshaense* Infection**

The β-GRP interference group and the control group were further cultured in soil also cultured with *M. pingshaense* to investigate the role of β-GRP in antifungal immune response. Compared with the control group (dsGFP and PBS), the knockdown of β-GRP significantly decreased survival rate (fold change >10 at day 4) (Fig. 5). This result indicated that β-GRP can regulate the immune response of *M. cribricollis* larvae to defend against fungal invasion. We also detected the expression level of antimicrobial peptide-related genes after silencing β-GRP in vivo. As shown in Fig. 6, the expression levels of defensin and lysozyme in the experimental group were strongly downregulated (fold changes were >2.6 and >1.5, respectively) compared with those in the dsGFP and PBS injected groups. This result indicated that β-GRP probably has the potential to activate the immune system to resist pathogen invasion.

**Discussion***

Insect β-GRP protein was first identified in *Bombyx mori* and was subsequently discovered in other insects (Dai et al. 2013). Bacterial and fungal cell wall components, including lipopolysaccharide, peptidoglycan, and β-1,3-glucan, are collectively referred to as PAMPs (Janeway 1989). Due to the strong specific affinity for β-1,3-glucan, upon binding with β-GRP, it activates the ProPO cascade immediately and then the immune system to prevent bacterial and fungal
invasion (Ochiai and Ashida 2000). β-GRP is an essential protein to recognize foreign pathogens. However, it’s the molecular characteristics of β-GRP and its biological immune function in *M. cribricollis* larvae remain largely unknown.

In the present study, on the basis of the RNA-seq result of *M. cribricollis* larvae we obtained previously, a β-GRP homolog gene of *M. cribricollis* larvae was firstly identified, and its immune function was characterized. We first obtained the full-length sequence of β-GRP through RACE-PCR. The pI of β-GRP protein was 6.17, which was similar to the β-GRP pl (6.18) from *Plutella xylostella* (Linnaeus) (Lepidoptera: Plutellidae), and both were predicted to be hydrophilic proteins (Chen et al. 2017). β-GRP/GNBP family members have glycoside hydrolase domain of the GH16 superfamily at the C-terminal (Boraston et al. 2004, Cantarel et al. 2009). In this study, β-GRP of *M. cribricollis* was predicted to contain conserved GH16 domains (Fig. 1). Insect β-1,3-glucanases are similar and evolutionarily related in sequence with recognition protein sequences, which play an important role in pathogen recognition (Genta et al. 2009, Bragatto et al. 2010). In this study, sequence comparisons and phylogenetic analysis results showed β-GRP acid sequence was lack of catalytic region, clustered into the same clade with other recognition protein. It was noteworthy that the recognition protein sequences from *A. gambiae* (GNBP-B1, ABU80032.1)
β-GRPs can bind to fungal β-1,3-glucan, activate Toll pathway and ProPO signaling, induce antimicrobial peptide production, and promote melanization cascade (Mishima et al. 2009). The expression of β-GRP gene in silkworm epidermis, fat body, and hemolymph increased under fungal infection (Ochiai and Ashida 2000). The expression level of β-GRP in Locusta migratoria manilensis (Meyen) (Orthoptera: Acrididae) significantly increased after the insects infected by Metarhabdum azidium (Hypocreales: Clavicipitaceae) (Zheng and Xia 2012). Similarly, after the infection with M. acridum, the expression levels of Tpβ-GRPe and Tpβ-GRPd in the fat body of the Thitarodes pui (Lepidoptera, Hepialidae) were significantly upregulated (Sun et al. 2018). Other studies have also confirmed that β-GRP is crucial in the immune mechanism of insects against fungal infection (Fabrick et al. 2004, Chen et al. 2017, Zhao et al. 2017). Consistent with the aforementioned results, our study also showed that the time-course profile of β-GRP gene from M. cribricollis was significantly upregulated after treatment with M. pingshaense and maintained a high level (Fig. 4). These results suggested that β-GRP was probably involved in immune response.

M. pingshaense is an effective entomopathogenic fungus of the family Clavicipitaceae that can be applied to pest control (Lovett et al. 2019). In present study, we used M. pingshaense as an exogenous pathogen to elucidate how β-GRP gene modulates the immune system of M. cribricollis larvae under fungal infection. We developed dsRNA interference technique to knockdown the expression level of β-GRP in vivo, cultured M. cribricollis in soil also containing M. pingshaense, and then monitored the survival rate of M. cribricollis. Compared with that of two groups (dsGFP and PBS treated), the mortality of M. cribricollis significantly increased. This result indicated that β-GRP had the potential to regulate the innate immune system of M. cribricollis to defend fungal infection.

Humoral immunity is the dominant barrier to protect the host from pathogen invasion by promoting the release of antimicrobial peptides (Diamond et al. 2009, Sheehan et al. 2018). To demonstrate the molecular mechanism underlying β-GRP resistance to pathogen invasion, we evaluated the transcription level of antimicrobial peptides genes defensin and lysozyme after β-GRP interference. After silencing β-GRP in vivo, the expression of defensin and lysozyme significantly declined. This result indicates that β-GRP might regulate the body’s antifungal immune response by inducing the expression of antimicrobial peptides.

In summary, a novel β-GRP of M. cribricollis was first characterized. The results suggest that this gene probably plays a crucial role to stimulate the expression of antimicrobial peptides in M. cribricollis larva against fungal infection. Our findings would enrich the understanding of β-GRP function in the innate defense immune mechanism of insects.

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Author Contributions

Bihuan Ye was in charge of experiments implementation, data analysis and manuscript writing. Haibo Li was in charge of data analysis and manuscript revision. Qiyuan Song and Jianjun Shen were in charge of the pathogenicity tests. Jinping Shu and Yabo Zhang were in charge of the sample collection. Youwu Chen was the author of the conception of the study and its design.

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