ISJ 14: 157-164, 2017 ISSN 1824-307X

RESEARCH REREPORT

Molecular cloning and characterization of High Mobility Group box（HMGB）gene from *Beauveria bassiana*- infected silkworm, *Bombyx mori*

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Accepted April 18, 2017

Abstract

The cDNA sequence of High Mobility Group box (HMGB) from *Bombyx mori* was cloned by rapid amplification of cDNA ends and submitted to GenBank (the accession number was JF969272). The full-length cDNA of *Bm*HMGB included 3015 bp, with five exons and four introns. It contained a 313 bp 5′ UTR, a 2114 bp 3′ UTR with a polyadenylation signal sequence AATAAA and a poly(A) tail. It encodes a 195- amino acid polypeptide with a predicted molecular weight about 22991.9Da and a theoretical isoelectric point 10.00. Phylogenetic analysis revealed that *Bm*HMGB is grouped in insect HMGB proteins. *Bm*HMGB was mainly expressed in hemolymph, cuticles, midgut and fat body by RT-PCR. In these tissues, the relative expression of *Bm*HMGB with *Beauveria bassiana* infected silkworm was higher than the normal ones by fluorescent quantitative real-time PCR. Therefore, *Bm*HMGB may play an important function in the response to *B. bassiana* infection of silkworms.

Key Words: *Bombyx mori; Beauveria bassiana;* High Mobility Group box protein; rapid amplification of cDNA ends; qRT-PCR

Introduction

High Mobility Group box (HMGB) proteins are one of the cytokines with diverse functions, exist at the intersection of foreign and endogenous molecules in all eukaryotes (Andersson and Tracey, 2011; Malarkey and Churchill, 2012; Choi *et al*., 2016). These proteins have one or multiple HMG-box, which is an unique and versatile DNA-binding domain. They can bind many substrates, such as different immunogenic nucleic acids, bended/loopped and unwinded DNA, damage associated molecular pattern (DAMP), Toll-like receptor (TLR) (Stros, 2010; Malarkey and Churchill, 2012). As DNA chaperones, they also influence many biological processes in chromatin from maintenance of genome integrity to transcription, such as DNA replication and repair, recombination, transcription, genomic stability, so they throw a critical vote in decisions of life and death (Gerlitz, 2009). HMGBs have another crucial role that they act as an endogenous immune signal to inform other cells that damage or invasion has occurred (Yang and Tracey, 2010). In the nucleic-acid-mediated innate immune responses, HMGBs act as an universal sentinels, that is all exogenous nucleic acids must bind firstly HMGBs, then be sent to specific pattern recognition receptors and activate the innate immune responses (Yanai *et al*., 2011). They also function as DAMPs, which can activate inflammatory and induce immune responses to protect against infection and promote healing after tissue damage in organisms (Manigrasso*et al*., 2014; Choi *et al*., 2016).

The silkworm, *Bombyx mori*, an important economical insects and model organisms of lepidoptera, only has the natural immune system. *Beauveria bassiana* is one of the major fungal pathogens for silkworms and can cause economic damages to the sericultural industry. During the silkworm defense pathogen-infected, immune recognition is the first step of the responses. In our previous studies, we found a different expressed gene *Bm*HMGB with recognized function (Hou *et al*., 2014). To further study it, we first cloned the full-length cDNA of *Bm*HMGB by rapid amplification of cDNA ends. Then the expression characteristics of *Bm*HMGB was analyzed in different tissues and at different time in the same tissue after *B. bassiana* infected. These results provide some useful information for further study of the immune recognition mechanism of silkworm to fungi infection.

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Materials and Methods

Silkworm and B. bassiana *strain*

In our study, the silkworm strain p50 and *B. bassiana* HN6 were provided by the Sericultural Research Institute of the Chinese Academy of Agricultural Sciences. The larvae of silkworm were reared with mulberry leaves at standard temperature and humidity until the newly exuviated fifth instar larvae for the experiments. HN6 were originally inoculated from the cadaver of the infected silkworm to potato dextrose agar (PDA), then isolated and cultured on PDA for about 10 days at 25 °C.

Inoculation of B. bassiana

Conidia of *B. bassiana* were scraped from PDA and diluted to 3×10^8 spores/ml using sterile distilled water (containing 0.01 % Tween-80). The newly exuviated larvae were immersed in the conidia solution for 15 s. The control larvae were immersed in sterile distilled water (containing 0.01 % Tween-80) for the same period. Then regulated temperature and humidity to 28 °C and 95 % RH, the larvae were reared in these conditions.

Collection tissues

The different tissues were collected from both the HN6-infected and control larvae at 8 h post-inoculation (hpi). First, hemolymph was collected, it was directly mixed with pre-joined Trizol reagent (Invitrogen) in an Eppendorf tube. Then the larvae were rapidly dissected to collect gonad, martensite, mid-gut, mid-silk gland, fat body and cuticles. These tissues from 10 - 15 larvae of fifth instar larvae were pooled as one sample. The tissues were quickly washed in diethylpyrocarbonate -treated PBS solution (137 mM NaCl, 2.68 mM KCl, 8.1 mM Na_2HPO_4, 1.47 mM KH_2PO_4, pH 7.4) and immediately frozen in liquid nitrogen before stored at -80 ℃refrigeratory. The collected time was 8, 10, 12, 15, 18, 24 and 36 hpi, respectively. The larvae hemolymph of 36 hpi was used to cDNA cloning.

Cloning Full-length cDNA and sequencing of Bm*HMGB*

Total RNA was extracted respectively from different tissues by using a RNIpure- total RNA rapid extraction kit (Beijing Boling kewei Biotechnology) according to the manufacturer's protocol. Then the degraded condition of tRNA and its concentration was determined separately. Subsequently the eligible tRNA from hemolymph of 36 hpi was used to synthesize the first-strand cDNA according to the protocol of PrimeScript™ RT reagent Kit with gDNA Eraser (Takara). Full-length cDNA of *Bm*HMGB was synthesized using the first-strand cDNA as templates and SMART™ RACE cDNA Amplification Kit (Clontech). The specific primers for 5' and 3' RACE were designed according to the EST sequence revealed in our previous study. 3'-RACE-ready cDNA was prepared according to the Clontech Kit protocol. The specific primer P1 (5'-CGTTGACTGACAATGAAGAAGCGAACAGTTG-3', 5'-TACTTATGTCAACGGACACTGAAGGTCGGA-3', 5'-CGTTGACTGACAATGAAGAAGCGAACAGTTG-3) and P2 (5'-CAACTGTTCGCTTCTTCATTGTCAGTCAACG-3') were separately used for amplification of 5'and 3' -end in the RACE reactions. The PCR amplification progress was: 95 °C for 1m; 28 cycles of 95 °C for 15 s, 68 °C for3 min; then 72 °C for 10 min. The PCR products were gel-purified and cloned into pMD19-Tsimple vector (Takara). The positive recombinants were selected by anti-Ampicillin after being transformed into *Escherichia coli* Top10, then PCR screening was done with M13 primers (F: 5'-TGTAAAACGACGGCCAGT-3', R: 5'-CAGGAAACAGCTATGACC-3') and then sequenced. The sequences of *Bm*HMGB were assembled with the obtained fragments from RACE and analyzed at the National Center for Biotechnology Information (http: // www. ncbi. nlm. gov/blast). The amino acid sequence of *Bm*HMGB was analyzed with the Expert Protein Analysis System (http://www.expasy.org/) and SMART program (http://smart.embl-heidelberg.de/). The work of multiple protein sequences and phylogenetic tree was employed by the Clustal W and neighbor-joining method in MAGE 6.06 software. Bootstrap trials were replicated 1000 times to derive the confidence value for the phylogeny analysis.

RT-PCR

RT-PCR method was used to detect the expression condition of *Bm*HMGB in different tissues of silkworm. 1μL (500 ng) tRNA, extracted from different tissues, was used as template to synthesize cDNA using the Prime Script ™ RT Reagent Kit (TaKaRa). The PCR reaction was performed to describe the expression in these tissues or not.

Quantitative real-time PCR (qRT-PCR) of Bm*HMGB*

The expression level of *Bm*HMGB in different tissues of silkworm was detected by qRT-PCR method. QRT-PCR was performed using 2 μl of diluted cDNA in each 20 μl reaction mixture according to the manufacturer's instructions of the SYBR Premix Ex Taq™ (TaKaRa). cDNA templates were synthesized from the hemocyte, cuticles, fat body and midgut of *B. bassiana* -infected and control larvae at 8, 10, 12, 15, 18, 24 and 36 hpi. Specific primers for *Bm*HMGB (F: 5'-ACAGTCTGAACTGCTTGATCCA-3', R: 5'-AATCAACCAGTGCTCCCCAA-3') and β-actin (an endogenous control gene, F: 5'-AATGGCTCCGGTATGTGC-3', R: 5'-TTGCTCTGTGCCTCGTCT-3') were designed by Primer Premier 5.0 software. Reactions were run in triplicate on a 7300 Sequence Detection System (Applied Biosystem). The thermal cycling parameters of qRT-PCR was: 95 °C for 30 s; 40 cycles of 95 °C for 5 s, 60 °C for 31 s; 95 °C for 15 s, 60 °C for 60 s, 95 °C for 15 s. Each tissue contained the control and affected sample at different times and its β-actin was run in the same plate. The relative expression levels of *Bm*HMGB gene were normalized using the Ct values obtained from the β-actin amplification run in the same plate. The mean value ± SD and $2^{-\Delta\Delta Ct}$ method were used to analyze the relative transcript levels of each time point.

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1     ACAATAAGCGCTGAAATCGTGTGCTCGTTTGTATTGAGAATTTTAGTTGTCGCAGAATCA
61    GTCAAATCAAGAAAGCGATCAAAACAATCAACAAGCGAACGGTATTCAACAGAGTCATCA
121   GTCGATCCAACAACAACAGCAGCAGGAGCAAAATCAAACTATTCAACAGCAACAAAGTCA
181   ATTACAACAGCAGTTGCAGCAACAACAGAATCTCCAGCAAGCTTTGCAACAGCAGAACCA
241   ATCTTTGCAGCAGTCGTTACAACAACAACAACAACAGCAGCAGCAAGAAGAGCAGCAACC
301   GACCCTGCAGCAGATGCTACAGCAGCATCAACAGCAGCAGCACCAAGGCTTACAACAAAC
1                   M   L   Q   Q   H   Q   Q   Q   Q   H   Q   G   L   Q   Q   T
361   TTTGCAGCAGACATTGCAAGTTAGTCAGGCTCAAGCCCAAGCAATAGCGCAAGCCCAAGC
      L   Q   Q   T   L   Q   V   S   Q   A   Q   A   Q   A   I   A   Q   A   Q   A
421   AGCCTTACAGCACCAAGTTGCTCAATCAATACAGCAGCAACAACAAACTCTACAAGAACA
      A   L   Q   H   Q   V   A   Q   S   I   Q   Q   Q   Q   Q   T   L   Q   E   H
481   CATTCAAGCTGTTCAGCAACAACAGATACAGGCAGCATTACAGAGGCAATCTGCCACTCT
      I   Q   A   V   Q   Q   Q   Q   I   Q   A   A   L   Q   R   Q   S   A   T   L
541   ACAGGAGCTGCAGCAACAGGCACAGCAGCAAGCTTTACAGCAAGCAACAGTAAATAAGGC
      Q   E   L   Q   Q   Q   A   Q   Q   Q   A   L   Q   Q   A   T   V   N   K   A
601   CAGGATGCCCCGTTCAAGGCCATACAACAAGCCCCGCGGTCGCATGACAGCTTATGCATT
      R   M   P   R   S   R   P   Y   N   K   P   R   G   R   M   T   A   Y   A   F
661   CTTTGTGCAGACGTGCCGAGAAGAACACAAGAAGAAATACCCTGATGTCAGTGTTATATT
      F   V   Q   T   C   R   E   E   H   K   K   K   Y   P   D   V   S   V   I   F
721   TGCAGCATTCTCGAAAAAGTGCGCAGAGAGGTGGAATACAATGTCGGAAAAAGAAAAACA
      A   A   F   S   K   K   C   A   E   R   W   N   T   M   S   E   K   E   K   Q
781   GCGGTTCCATGAGATGGCTGAACAGGAC AAGCATCGATTCGACTTGGAGATGCAGAACTA
      R   F   H   E   M   A   E   Q   D   K   H   R   F   D   L   E   M   Q   N   Y
841   TGTACCACCAAAGGACATGAAGGTCAGAGGGCGAAAGAGGCAGCAGTATGAAAGACCCTAA
      V   P   P   K   D   M   K   V   R   G   R   K   R   Q   Q   Y   E   R   P   *
902   TGCACCAAAGCGCTCGCTATCAGCATTCTTTTTGTTTTGCAACGATGAACGTTCGAAGGT
962   GAAAGCTGGCAACCCAGAGTACACCATGGGCGATATTGCGAAGGAACTCGGCAGACGTTG
1022  GGCGGCCGCTGATCCGGAGACTAAGGCTAAATATGACGCGCTATCTGAAAAAGACAAGGC
1082  GCGATATGATAGGGAAATGACAGCGTACAAGAAAGGTCCGTTAGCTCAGCCGCCGCCACA
1142  GCCGCCTGTCGTCCCCGCCATCGAGGACGAGGGCGGAGACTTCGATGCAGACGAAGAATA
1202  CAATTGAAATAATGTGATCGCTGTCGATACAGCCTAAAATCAGGACAGAGAGATTTAACT
1262  CGATTTTGAAGATGAAAGTGCGCGACAGTCTTTCCCCCTTTTTCCAGCCAAATGTATAAC
1322  GCAATTGCTGTATACATTACTGATTTGTTTTGTTCATTATTCAGGGGATGATTTATTTTT
1382  GTGCTGCGGGTTTGGGGAGCACTGGTTGATTGAGTTTAATAGAAATTGTAATCTTGTTTT
1442  TGTTAGAGGGGGGGTCGTCGTCGTCATTCAAGGAAGGGTCGTAAATTTAAGGAAGCGTTT
1502  TGACGTACGATCAGGTGTCAGAGAGGCTGGGTCTTTGAACGAAAGATTGAGGAGTGAATC
1562  TTCTGTTCTGTAGCGTGTTATAGTTTTTTACAAATTTAATTCTGATTCACAGATTTTACT
1622  TATACTTTCAAATACTCAAAATATTTTTGTACGATTTTTTGTTTGTCATCTGAGAAATGA
1682  GGTGTCAAATAGTTTTATTTACAGCTTAAAAAAACGGCCAAACACCTATGTCGTTTTAAA
1742  AAAAAAACCTCTAATTTTATGTATTCTTCAACAAATAAATGATTATCTTTAGTTCTCAGA
1802  TGACGAATGTTGAACAGATTTTGAATGACAAAAATTTTAACATGAAAATGACTTTTAATT
1862  GTTGCAAACTTTTGTAAACGAAACTGACGTTATTTTATGATCTTACGTTGACTGACAATG
1922  AAGAAGCGAACAGTTGATTTTTTTTATTTTTCTTGATACGAATTTCTTCGAGATACAAGG
1982  AAAAAAGTAGACAATATGACAATTGGCAGTTAAAAAAAAATTGTCATTCTCCCGCCAAAA
2042  TATCGTACCATGAAAGTAAAATCAAAAAGTGTCATGGCGCCCAAAACATGTCTTGCGTCT
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2102 ACTTATGTCAACGGACACTGAAGGTCGGAAATGGTGTGGNCCCTGTGGTTTAATAAAATC
2162 GTAATTACAAGCAATATTATCAGGAACAAATCGATACAGACGTTTTCAGTATCACGTATA
2222 ATGTAAGTCAAAAAGTATTTCTTATTAAGTTAGCATAGACGTAAGGAGGTCGGGAGCGAA
2282 TGTATTGTTGTTGTTTTGTGTCGATTATGTATAGTTAAATTATATTCCTTTATTAAATAT
2342 GTACTTTTGTTTGATTTCGATATAATTTATAAATCGCGTTTAGTTAAAATATTAAGTTAC
2402 GAAATTTTATATTTTGTGAATTGTTTGTTTAGTGAGTCTGTCTCGCGCCATTTCTGTTTG
2462 ATGCATGCATACCAGCGATGTGTTTTTGTACATAAAACGACTGCAACCCGTGATAGTACT
2522 TAATAGTAGCTGCGCTAGGTACTACAATATAGGTTATGTATATGATTTCTTCTGAAAAAT
2582 TCAATTCCAAATGAAATTAATTCGCTCATTGTTTTTCGTTTACTGTCTGATTGTTCGTTT
2642 GAAATCCGTTTACGTTTTCAAAGAATATAATTTCAAGTATTTTGAAAGAATAAACGAATA
2702 GTCCTCTTCTGATAAAACATACGAATGATGGTGTTCAAACCATTTGATATGCTAAGTTCT
2762 TTTTAAGTGCATAATAATGCATAACTTGAAACACACGTGTCGGCGTCTTTACTCGGCAGA
2822 AGACTGTTTAAATATCTTTTTCGAACGGACATTACCGTAAATATAATATGTATGTACCAG
2882 TTTTTTTTAGATTATTAAAAAAAACACGAGATTTGACCCCGCTTGGAGAGGCAAAGCGAT
2942 GAAGTGATTTTAAGTTGTATCGAAAAGGGCAATTGTTTTAAATTTGTAAAACGCTCACTG
3002 TACCAAAAAAAAAA

Fig. 1 The full-length nucleotide sequence information of *Bm*HMGB. The initiation and stop codons are boxed, the trailing signal (AATAAA) is underlined. Black area is the HMGB domains. Green area is the coiled coil region. Red word is N-myristoylation site (No. 12-17).

Results

Analyse of the Bm*HMGB full length and structure*

The full length cDNA of *Bm*HMGB protein was obtained by RACE, then sequenced, spliced and uploaded to GenBank (Accession no. JF969272). It contained a 313 bp 5'untranslated region (UTR), a 2114 bp 3' UTR and a 588 bp open reading frame encoding a 195 amino acids protein with a predicted molecular weight about 22991.9 Da and a theoretical isoelectric point 10.00 by Protparam software (https: //www.expasy) (Fig. 1). A putative polyadenylation signal AATAAA was detected in the 3'UTR 310 bp upstream from the poly(A) tail. At the No.106-178 amino acid sequences of the polypeptide chain, there was a *HMGB* domain which is a DNA binding site domain and this protein belongs to the HMGB family. There was a coiled coil domain at the No. 41 - 96 amino acid sequences (Fig. 2). The sequence contained a N-glycosylation site (No. 12-17), three Protein kinase C phosphorylation sites (No. 120-122, 140-142, 151-153) and five Tyrosine kinase phosphorylation sites (No. 52-55, 75-78, 120-123, 149-152, 151-154) (https://blast. ncbi. nlm. nih. gov, http://smart.embl-heidelberg.de) (Fig. 1). Analysis of the amino acid sequence by TMpred revealed that, this protein had no remarkable transmembrane region.

Aligning the full cDNA of *Bm*HMGB to the genome sequence of *B. mori*, it was contained on the Bm_scaf1_contig476, this contig has 44217 bp and the accession number is BABH01000476.1. It showed that *Bm*HMGB cotained five exons and four introns and each exon-intron boundary conforms with “GT-AG” rule (http://www.expasy.org/).

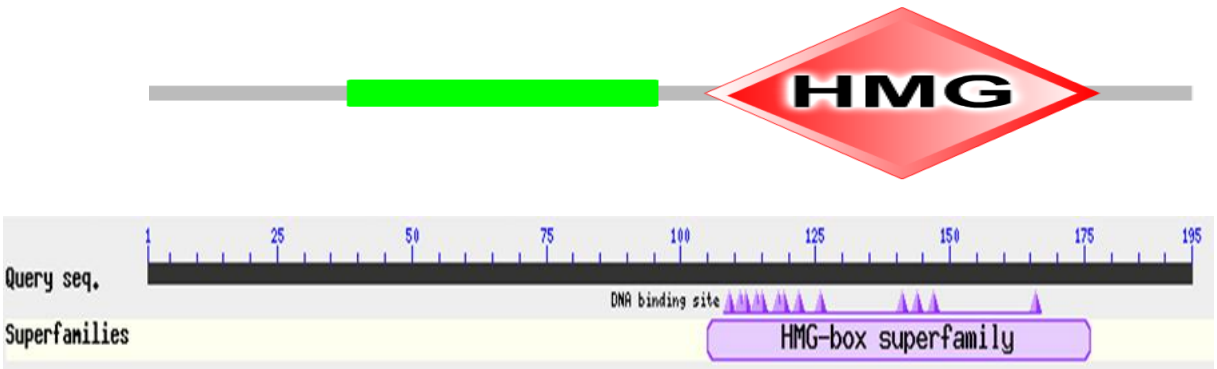

Fig. 2 Domain architecture analysis of *Bm*HMGB（Green area is coiled coil region）

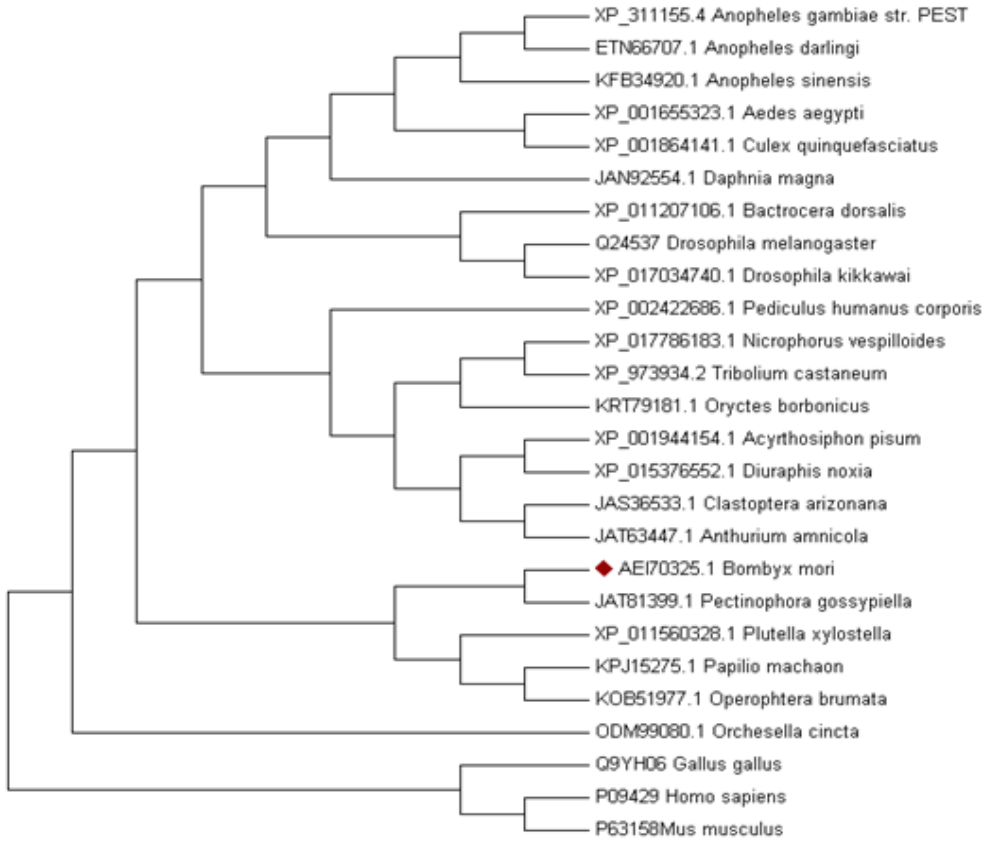

Fig. 3 Phylogenetic tree of *Bm*HMGB and other homologous protein.

The analysis of homologous sequences

The phylogenetic tree was constructed according to the amino acid sequences of HMGB homologous genes by MAGE 6.06 (Fig. 3). The results showed two separate clusters of vertebrate and invertebrate HMGBs, *Bm*HMGB was grouped in insect HMGBs of the invertebrate group. In the invertebrate group, some insects which should live in water for some period, including *Anopheles gambiae, Culex quinquefasciatus,* Aedes aegypti which showed high identity (75 %, 69 %, 66 %) with *Bm*HMGB.

Confirmation of infection

At approximately 48 hpi after *B. bassiana* spores infected the silkworm larvae, oily spots appeared on their body, which is one of the typical symptoms of the white muscardine disease. All of the infected larvae died in 60 hpi. The disease was further confirmed by the observation of hypha in hemolymph under a microscope and the appearance of the symptom of muscardine.

Expression of Bm*HMGB in different tissues*

The test result of RT-PCR revealed that *Bm*HMGB was expressed in hemolymph, cuticles, malpighian tubule, mid-gut, fat body, mid-silk gland, post-silk gland, and gonad. In contrast, it had lower transcript level in gonad and malpighian tubule. It mainly expressed in hemocyte, cuticles, fat body and midgut (Fig. 4).

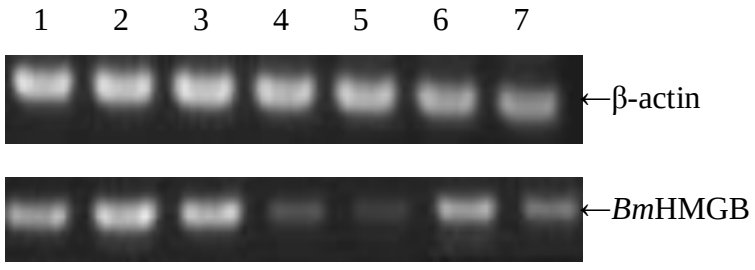

Fig. 4 The expression of *Bm*HMGB in different tissues. 1. hemocyte 2. cuticles 3. fat body 4. malpighian tubule 5. gonad 6. midgut 7. mid-silk gland

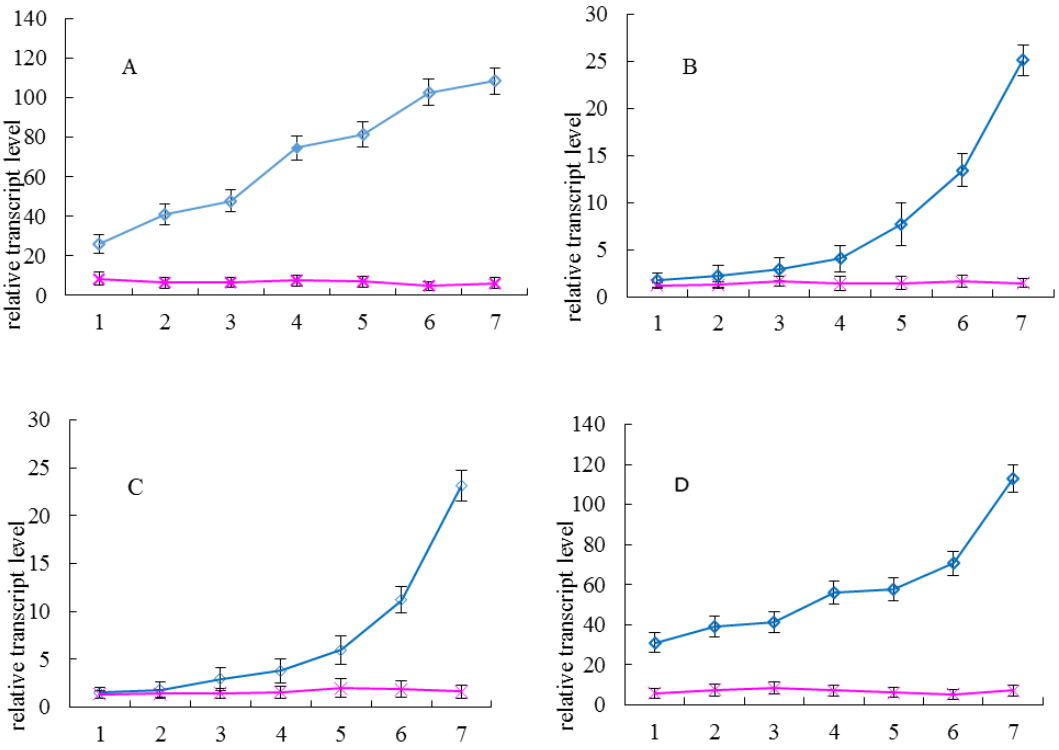

Fig. 5 The relative expression level of BmHMGB in normal tissues and *B. bassiana*-infected tissues (A, B ,C, D are hemocyte, cuticles, fat body and midgut, separately. ◊ is infected tissues, × is normal tissues. 1-7 is different time points post-inoculation, namely 8, 10, 12, 15, 18, 24 and 36 h). The relative expression levels of Bm*HMGB* gene at each time points were normalized using the Ct values obtained from the β-actin amplifications run in the same plate. All samples were tested in triplicate. The mean value±SD was used for the analysis of relative transcript levels for each time point using the ΔΔCt method. The infected and control individuals are blue and red, respectively.

Expression difference of Bm*HMGB in control and B. bassiana-infected tissues of silkworm*

According to the result of RT-PCR, the tissue-specific expression of *Bm*HMGB transcripts at different times was examined by qRT-PCR (Fig. 5). Between the normal and infected tissues, the expression variety of *Bm*HMGB was significantly different. In the control tissues, there was almost no variety in different times. While in the infected ones, there was significant variation. The relative expression of *Bm*HMGB in the infected tissues was higher than the control ones, especially with the extension of the infected time. For example, at 24 hpi, the relative expression levels in the fat body was 6.11-fold, in the cuticles was 8.34-fold, in the midgut was 10.31-fold and in the hemolymph was 21.71-fold.

Discussion

Beauveria is an important entomogenous fungi with a very broad host range, high pathogenicity and adaptability. Some *Beauveria* species have a wide use in the biological control of agricultural and forestry pests, but some threaten severely to silkworm and other economic insects. So far, the mechanism of the silkworm how to recognize fungi and initiate immune responses against *B. bassiana* infection is still poor. In our previous study, we screened and identified *Bm*HMGB, an up-regulating gene which encoding High Mobility Group Box protein (Hou *e al*., 2011, 2014).

HMGBs are one kind of multifunctional proteins, architectural chromatin nuclear proteins which have been implicated as a mediator or alarmin of inflammatory and autoimmune diseases in multicellular organisms (Voll *et al*., 2008). They act as an innate alarm and trigger in the initiation of host defenses or tissue repair, and they may be an integrators of signals that maintain the peace and stress, life and death (Bianchi and Celona, 2011). They participate the activation of TLRs and other cytosolic receptors in immune response (Tian *et al*., 2007; Kazama *et al*., 2008). In these activation progress, HMGBs act as an universal sentinels, that is all immunogenic nucleic acids of the exogenous microorganisms first be promiscuous sensed and binded by HMGBs, then be discriminative sensed by specific pattern recognition receptors and activate immune responses of host. HMGBs shuttle continuously between nucleus and cytoplasm to hunt the invading nucleic acid of virus (Bonaldi *et al*., 2003). They bind to nucleic acid without specific sequences, so it is very difficult to evade HMGBs surveillance for virus (Bianchi and Celona, 2010). If

HMGBs knockout, the activation of TLR3, TLR7 and TLR9 by their cognate nucleic acids will be severely impaired so the cells can't sense the invasion of pathogens (Wang, 2009). Now they have attracted considerable attention due to they can be recognized by various cell surface receptors and their potent pro-inflammatory activities associated with diverse and major human diseases (Andersson and Tracey, 2011; Venereau *et al*., 2012; Yang *et al*., 2015; Eunjin *et al*., 2016).

HMGB proteins include single or multiple HMG boxes. B box is a domain of induced inflammatory response and can efficiently activate macrophages to release tumor necrosis factor and other cytokines, while box A antagonizes it (Li *et al*., 2003; Andersson and Tracey, 2011). When the host cells are exposed to pathogen-derived molecules, HMGBs can be actively secreted by immunological cells, or passively released by injured, apoptotic or necrotic cells (Andersson *et al*., 2000; Andrassy *et al*., 2008; Tsung *et al*., 2005). The passively released HMGB can induce an inflammatory response, promote tissue repair and angiogenesis (Yun *et al*., 2013).

The innate immunity of insect emerges as the defensive frontline that coordinates the interaction between host and pathogen. Insect can use apoptosis as another defense strategy, while pathogen synthesize anti-apoptotic proteins like p35 to prevent host cell death against its defense (Narayanan, 2004). So, it is important to understand the interaction between host and pathogen, the defensive mechanisms about host response infection and the anti-infective mechanisms of pathogen. These are important for better controlling pathogen spread, researching efficient utilization of entomopathogenic fungi and developing new drugs. In our study, the relative expression of *Bm*HMGB in the *Beauveria* -infected tissues were higher than that of the control ones, especially in the hemocyte and midgut. Among of these tissues, there was a remarkable difference in the hemocyte. at 24 phi, the relative expression of the infected-hemocyte was approximately 21.71-fold of that in the normal one. We suppose that after the invasion of *B. bassiana*, the response of natural immunity of host was activated causing the immune cells to become active, infected and apoptotic cells passively released HMGBs which led the transcript level of *Bm*HMGB in infected tissues was increased. This may be one of the reasons that led the dysfunction of host and eventually caused its death.

In summary, during *Beauveria* infect the silkworm, a series of physiological and pathological changes and immune responsive reactions take place in the host. As a corresponding result, the transcript levels of some related genes also change. According to our study, we suppose that *Bm*HMGB may play an important role in the interaction of silkworm and *Beauveria*. Further functional experiments of *Bm*HMGB should be taken.

Acknowledgments

This work was supported by the National Natural Science Foundation of China (Grant No. 31672358) and the Natural Science Foundation of JiangSu Province (BK20161364).

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