RESEARCH

Identification and Expression Analysis of *QM-Like* Gene From *Spodoptera litura* After Challenge by the Entomopathogenic Fungus *Nomuraea rileyi*

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Subject Editor: Ann Fallon

J. Insect Sci. 14(200): 2014; DOI: 10.1093/jisesa/ieu062

ABSTRACT. A partial sequence of QM homologue was isolated from a *Spodoptera litura* fatbody suppression subtractive hybridization library. The full-length *Spodoptera litura* QM (*SpLQM*) cDNA of 838 bp contains a 5′ untranslated region (UTR) of 112 bp, a 3′ UTR of 66 bp, and an open reading frame of 660 nucleotides coding for a 219 amino acid peptide with a molecular weight of 25.5 kDa. Analysis of SpLQM sequence revealed the presence of characteristic motifs, including the ribosomal protein L10 signature and SH3-binding motif. Multiple alignment analysis revealed that SpLQM shares an overall identity of 57.1–99.1% with other members of QM family. Phylogenetic analysis confirmed that SpLQM is closely related to other insect QMs. Analysis of the tissue expression pattern showed that the *SpLQM* mRNA was expressed in all tissues tested, with highest levels measured in hemocytes, followed by fat bodies. Upon *Nomuraea rileyi* challenge, *SpLQM* showed significant upregulation in fat bodies and hemocytes, while slightly upregulation in midguts. The results suggest that *SpLQM* might play an important role in the innate immunity of *S. litura* in response to *N. rileyi* infection. SpLQM was also successfully overexpressed in *Escherichia coli,* and the recombinant fusion protein SpLQM-His has a molecular weight of 32 kDa.

Key Words: *QM* gene, gene expression, innate immunity

Spodoptera litura is an economically important polyphagous pest worldwide, especially in China, India, and Japan, causing considerable economic loss to many vegetable and field crops (Pan et al. 2005). Prevention and control currently has mainly depended on various classes of chemical insecticides including carbamates, pyrethroids, and organophosphates (Liburd et al. 2001). Widespread and continuous use of these chemical insecticides have caused insect resistance and environmental problems (Ahmad et al. 2007). Microbial insecticides can provide an alternative, more environmentally friendly option to control this insect pest. *Nomuraea rileyi* is one of the dimorphic hyphomycetes that can cause epizootic disease in various insects, especially in noctuidae insects such as Spodoptera, Pseudoplusia, Trichoplusia, Plutella, and Rachiplusia (Boucias et al. 1984, Srisukchayakul et al. 2005, Sandhu et al. 2011). Due to *N. rileyi* strong pathogenicity against *S. litura*, it has been used in insect management. However, the molecular mechanisms of immune defense in *S. litura* against *N. rileyi* are obscure.

QM gene was originally identified in the nontumorigenic Wilm's cell line by subtractive hybridization and then proposed to be a tumor suppressor due to the higher expression levels in nontumorigenic Wilm's tumor microcell hybrid cells than in the tumorigenic parental cell line (Dowdy et al. 1991). Since then, highly conserved *QM* homologues were cloned from various organisms, including vertebrates such as mammals (Dowdy et al. 1991) and fish (*Ctenopharyngodon idellus*; Yi et al. 2005), invertebrates such as insects (*Bombyx mori*; Hwang et al. 2000) and shrimp (*Penaeus japonicus*; Xu et al. 2008), and plants (Kashmir et al. 2009). Several studies have demonstrated that overall function of *QM* is critical to cell viability and confirmed its roles in cell growth, differentiation, and apoptosis (Nguyen et al. 1997, Green et al. 2000, Lillico et al. 2002, Ramses et al. 2003).

QM gene encodes for the ribosomal protein L10 and is most likely involved in a late step of the 60S subunit assembly to form 80s ribosome (Dowdy et al. 1991, Nguyen et al. 1998). Deletion of *QM* homolog GRC5/QSR1 in yeast was lethal, and mutation of GRC5/QSR1 resulted in a defect in protein synthesis, growth and cell division arrest, abnormalities of actin cytoskeleton, and mitochondrial respiration (Tron et al. 1995, Koller et al. 1996). Both human *QM* and chicken homolog of *QM/Jif-1* was reported to interact with the transcription factor c-Jun and thus inhibited c-Jun to activate genes containing AP-1 binding sites (Monteclaro and Vogt 1993, Stanbridge et al. 1994). Besides, *QMs* participates in biological responses to various external stimulations in different organisms. Chen et al. (2006) showed that the expression of tomato *QM-like* protein(*tQM*) in *Saccharomyces cerevisiae* protected yeast cells against oxidative stress (from H_2O_2, paraquat, and heat) by regulating intracellular proline levels. In *Arabidopsis*, *QM* homolog rpL10 was found to be involved in a defense strategy of plant cells against virus mediated by NIK1 (Carvalho et al. 2008). Likewise, *QMs* were proved to regulate the activity of phenol oxidase in response to virus infection in virus-resistant shrimp (Xu et al. 2008). In a recent study, significant upregulation of the *QM* gene from disk abalone was found in gill of disk abalone, but not in hemocytes upon bacterial and Viral Hemorrhagic Septicemia Virus (VHSV) challenge, suggesting it could respond to and facilitate a defensive effect against pathogenic infection (Oh et al. 2010).

In this study, a *QM-like* gene (designated as *SpLQM*) was isolated by fat bodies suppression subtractive hybridization (SSH) library from *N. rileyi*-challenged *S. litura* larvae. We sought to define potential innate immune function of *SpLQM* upon *N. rileyi* challenge.

Materials and Methods

Preparation of Conidial Suspension of N. rileyi. *N. rileyi* strain CQNr129 was provided by the Genetic Engineering Center of Chongqing University. The fungus was cultured on SMAY medium (abourand-maltose agar fortified with 1% yeast extract) at 28°C for 10 d. Conidia were collected and suspended in sterilized 0.05% Tween 80 solution at 1×10^7 spores/ml.

Immune Challenge of S. litura. *S. litura* larvae were reared on artificial diet at 27±1°C and 75±5% relative humidity (RH) with a photoperiod of 16:8 (L:D) h. Fourth instar healthy larvae were used in this experiment. In the experiment group, larvae (n=50) were injected with 5 µl conidial suspension, whereas others (n=50) were injected with 5 µl

of sterilized 0.05% Tween 80 solution without spores as the blank control. The larvae (10 larvae per group) were reared separately under the same conditions as above after treatment. Meanwhile, 10 untreated larvae were separately reared as a normal control. About 2 ml hemolymph was collected from each treatments of *S. litura* by cutting pleopod at 0, 6, 12, 24, and 48 h postinjection (hpi). The hemolymph was then centrifuged at 12,000 × *g* at 4°C for 5 min immediately to isolate the hemocytes. Other tissues such as fat bodies and midguts in both treatment and control groups were also dissected for RNA extraction at 0, 6, 12, 24, and 48 hpi.

To study encroach process of spores in hemocoel after *N. rileyi* injection, 20 µl hemolymph was collected from larvae at 24, 48, and 72 hpi or in normal control group, respectively; slides were prepared to observe under optical digital microscope (Motic, China).

Gene Cloning of SpLQM From S. litura. Total RNA was extracted from the fatbody of fourth instar larvae using Trizol reagent (Invitrogen, Carlsbad, CA, USA) following the manufacturer's instructions. First-strand cDNA was synthesized using the primer oligo-anchor CDSIII and SMART II oligonucleotide with SuperScript II Reverse Transcriptase (Invitrogen) following the manufacturer's instructions.

The gene-specific primers for 3′ rapid amplification of cDNA ends (3′-RACE) and 5′-RACE were designed based on the Expressed Sequence Tag (EST) of *QM,* while adapter primers were synthesized according to the instructions of SMART RACE Kit. 3′-RACE was performed with CDSIII and QMPF in the following conditions: denaturation at 94°C for 1 min, followed by 30 cycles at 94°C for 4 min, 55°C for 30 s, and 72°C for 30 s. A final extension step was conducted at 72°C for 10 min. A secondary nested polymerase chain reaction (PCR) was then carried out with CDSIII and QMPF using the 50× diluted primary PCR product as the template in place of the cDNA in the same conditions as the first round, except the annealing temperature at 60°C. Similarly, 5′-RACE was performed with 5′ PCR primer and QMPR in the following conditions: denaturation at 94°C for 4 min, followed by 30 cycles at 94°C for 30 s, 60°C for 30 s, and 72°C for 1 min. A final extension step was conducted at 72°C for 15 min. A secondary nested PCR was then carried out with 5′ PCR primer and QMPR1 using the diluted primary PCR product as the template according to the same conditions as the first round, except the annealing temperature at 55°C. Both the 5′-RACE and 3′-RACE products were cloned into pMD19-T vector (TaKaRa Bio Inc., Shiga, Japan) and sequenced. The full length of *QM* of *S. litura* was also obtained by overlapping the two fragments. To confirm the assembled cDNA sequence from overlapping PCR products, the entire coding regions of QM gene were amplified by PCR with the forward and reverse primers QMTF and QMTR. All primer sequences mentioned above are illustrated in Table 1.

Bioinformatics Analysis of SpLQM. The open reading frame (ORF) of *SpLQM* was identified using the National Center for Biotechnology Information ORF Finder (http://www.ncbi.nlm.nih.gov/gorf/gorf.html). Characteristic domains or motifs were identified using PROSITE database at ExPASy proteomics server (http://ca.expasy.org/; Bairoch et al. 1997). Signal peptide was predicted using signalP (Nielsen et al. 1997) prediction. The deduced SpLQM and other known QM sequences retrieved from GenBank were aligned with CLUSTAL W (http://www.genome.jp/tools/clustalw/) at default setting. Secondary structures of deduced amino acids sequences were predicted by SOPMA. A phylogenetic tree was constructed using MEGA version 4.1 (Tamura et al. 2007) from CLUSTAL W alignments according to the neighbor-joining method with a bootstrap test calculated with 1,000 replicates.

Genomic Structure Analysis of SpLQM. To analyze genomic organization of *SpLQM*, genomic DNA was extracted from the fatbody of fourth instar larvae as a template using a Genomic DNA Purification Kit (Omega, Bio-tek, Guangzhou, China) according to the manufacturer's instructions. Two gene-specific primers (JQMF1-JQMR1 and JQMF2-JQMR2) were designed based on the cDNA sequence. The PCR reactions were performed as follows: 94°C for 5 min, followed by 35 cycles of amplification (94°C for 30 s, 60°C for 30 s, and 72°C for 4 min 30 s). Cloning and sequencing of these PCR products were carried out as described above. Exons and introns were identified by comparing and analyzing the cDNA and genomic DNA sequences of *SpLQM*.

Tissue Expression of SpLQM in S. litura. To analyze the tissue expression of *SpLQM* in larvae, RNAs were isolated from various tissues, including head, cuticles, fatbody, midgut, malpighian tube, and hemocytes, respectively. The extracted RNAs were treated with RNase-free DNase (Fermentas, Vilnius, Lithuania) and verified by PCR using *β-actin* gene-specific primers and RNA as templates to exclude the possibility of genomic DNA contamination. Then, total RNA (2 µg) was used to synthesize first strand cDNA using the Revert Aid First Strand cDNA Synthesis (Fermentas) following the manufacturer's instructions.The resulting cDNAs were used as templates for the reverse transcriptase-polymerase chain reaction (RT-PCR) and quantitative real-time RT-PCR (qRT-PCR) with the *SpLQM*-specific primers (DQMF and DQMR). *β-actin* was amplified for internal standardization with the primers DactinF and DactinR. The primers used for RT-PCR and qRT-PCR were showed in Table 2. The PCR reactions were performed as follows: 94°C for 30 s, followed by 25 cycles of amplification (94°C for 30 s, 58°C for 30 s, and 72°C for 30 s) using 1 µl of first-strand cDNA in each 25 µl reaction mixture. Equal amounts of amplified products were separated by 2% agarose gel electrophoresis and stained with ethidium bromide. qRT-PCR was performed on Thermal Cycler of CFX96 (Bio-Rad, USA) using 0.5 µl of first-strand cDNA in each 25 µl reaction mixture with iQ SYBR Green Supermix (Bio-Rad) following manufacturer instructions. The thermal cycling profile consisted of initial denaturation at 95°C for 10 min and 42 cycles at 95°C for 15 s, 58°C for 30 s, and 72°C for 30 s. The specificity of PCR amplification was determined by constructing a melting curve after the PCR amplification. Each sample was processed in triplicate. The calculated relative expression level of each gene was compared

Table 1. The primers for RACE and genomic structure analysis of *SpLQM*

Primer name	Primer sequence 5′→3′	Description
CDS III	ATTCTAGAGGCCGAGGCGGCCGACATG-d$(T)_{30}$VN	3′ RACE universal adaptor primer
SMART II oligonucleotide	AAGCAGTGGTATCAACGCAGAGTACGCGGG	5′ RACE universal adaptor
5′ PCR primer	AAGCAGTGGTATCAACGCAGAGT	5′ RACE universal adaptor primer
QMPF	TCTACGTCTCCAAGAAATGGG	3′ RACE specific primer
QMPR	CTAGACGACCGTCCTCACGC	5′ RACE specific primer
QMPF1	GCGTGAGGACGGTCGTCTAG	3′ RACE nested specific primer
QMPR1	CCCATTTCTTGGAGACGTAGA	5′ RACE nested specific primer
QMTF	TGCTAAAGCGTGTTGTGTGA	Full-length cDNA verification primer forward
QMTR	ACGCTCGTACTTGGTGAAGC.	Full-length cDNA verification primer reverse
JQMF1	TTGCTAAAGCGTGTTGTGTG	Genomic sequence primer forward
JQMR1	ACGACAGAACCGTGATTTAG	Genomic sequence primer reverse
JQMF2	CTAAATCACGGTTCTGTCGT	Genomic sequence primer forward
JQMR2	TCATGGTATAATTATTGATGTTAAAGA	Genomic sequence primer reverse

Note: V = A,G,or C; N = A, G,C,or T.

Table 2. The primers for RT-PCR and qPCR analysis in this test

Gene	Name	Sequence 5′→3′	Product length (bp)	GenBank accession
SpLQM	DQMF	TCTACGTCTCC AAGAAATGG	78	JX134107
	DQMR	CTAGACGACCGTCCTCACGC		
ß-actin	DActinF	TGAGACCTTCAACTCCCCCG	178	DQ494753
	DActinR	GCGACCAGCCAAGTCCAGAC		

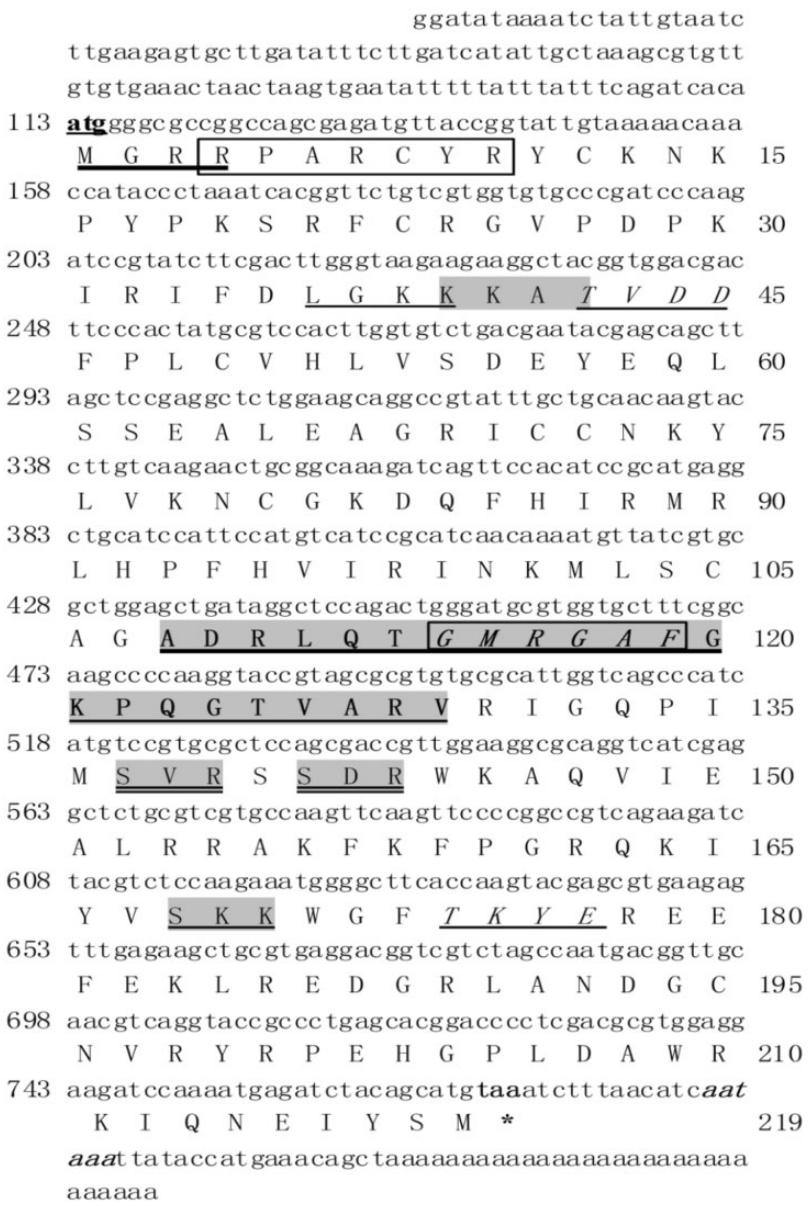

Fig. 1. The full-length nucleotide sequence and deduced amino acid sequence of *SpLQM*. The asterisk reveals the stop codon, and the polyadenylation signal (aataaa) is in bold italic. A series of motifs in putative SpLQM polypeptide are in shadow, including three protein kinase C phosphorylation sites SDR (141–143, SVR (137–139) and SKK (168–170), two caseinase II phosphorylation sites TDFD (179–182) and TKYE (174–177), an N-acylation site GMRGAF (114–119), and two acylamidation sites MGRR (1–4) and LGRK (36–40). In addition, a ribosomal protein L10 signature (108–129) is bold and shadowed, and an SH3-binding motif RPARCYR (4–10) is boxed.

with respective expression levels of head to determine tissue-specific expression.

Induction of SpLQM Expression Upon N. rileyi Challenge. To investigate the transcriptional changes of *SpLQM* in response to *N. rileyi* infection, the tissues of hemocytes, fat bodies, and midguts from challenge and unchallenge larvae were collected. Total RNA extraction, first-strand cDNA synthesis, the primers and the thermal cycling procedure of qPCR were the same as described above. qRT-PCR was performed using 1 µl of first-strand cDNA in each 25 µl reaction mixture. Each sample was processed in triplicate. *β-actin* was still amplified as a normalization. The relative expression level of the gene was determined by the Livak ($2^{-\Delta\Delta CT}$) method (Livak and Schmittgen 2001).

Recombinant Expression, Purification, and Verification of SpLQM. The coding sequence of *SpLQM* was amplified with Pfu DNA

polymerase (TaKaRa, Japan) using the primers BQMF: 5′-TTA GGATCC (BamHI) ATGGGGC GCCGGCCAGCGAGATGTT-3′ and BQMR: 5′-GGGAAGCTT (HindIII) CATGCT GTAGATCTCAT TTTGGA-3′ (where the underlined characters indicated restriction enzyme sites). The PCR product was ligated into pMD19-T simple vector (TaKaRa) and then transformed into *Escherichia coli* (DH5α strain). The plasmid pMD19-T-SpLQM was digested with BamHI and HindIII, and then the purified fragment was subcloned into the pET30a (+) expression vector (TaKaRa). After sequenced confirmation, pET30a (+)-SpLQM was transformed into *E. coli* BL21 (DE3 pLYsS strain) for protein expression. A positive clone was shaking cultured in Luria-Bertani medium supplemented with kanamycin (50 μg/ml) at 37°C. When OD_{600} reached 0.6, the culture was induced with isopropyl 1-thio-ß-D-galactoside (IPTG; final concentration of 0.6 mM) and further cultured for another 10 h at 17°C and then bacterial cells were collected by centrifuging the culture at 8,000 × *g* for 15 min.

Cells expressing the SpLQM protein were resuspended in denaturing lysis (8 M urea, 100 mM NaH_2PO_4, 100 mM Tris·Cl, pH 8.0) and subjected to three rounds of freezing or thawing by alternating the tubes between −70 and 37°C, and finally sonicated the cells to clarification on ice. The cell lysate was centrifuged at 13,000 × *g* for 20 min and the clarified supernatant was subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). The specific bands were excised and purified from the gel with a sterile scalpel according to the protocol of PAGE gel extraction Kid (Sangon, Shanghai, China). The extraction samples were analyzed by Western blotting with mouse anti-His-Tag antibody (Zoonbio, China) as primary antibody.

Statistical Analysis. The *SpLQM* expression level in different larvae tissues and between controls and challenged samples were analyzed by one-way ANOVA, followed by Duncan's Multiple Range test using the SPSS 17 program. Results of real-time quantitative PCR () were given as the means ± SE mean (SEM). Single asterisk (*) indicated the statistically significant level at $P < 0.05$, whereas double asterisk (**) displayed the statistically highly significant level at $P < 0.01$.

Results

Gene Cloning and Sequence Analysis of SpLQM cDNA. Based on ESTs database from the fatbody SSH library, the full-length *QM* was obtained by 5′RACE and 3′RACE (GenBank JX134107). The predicted *S. litura* QM amino acid sequence showed high homology to QM family members, and therefore it was designated as the *QM-like* gene from *S. litura* (*SpLQM*). The full length cDNA is 838 bp, including 112 bp 5′ untranslated region (UTR), 660 bp ORF encoding 219 amino acid proteins, and 66 bp 3′ UTR with a polyadenylation signal (AATAAA; Fig. 1). Deduced SpLQM protein has a molecular weight of 25.6 kDa and a theoretical isoelectric point of 10.0.

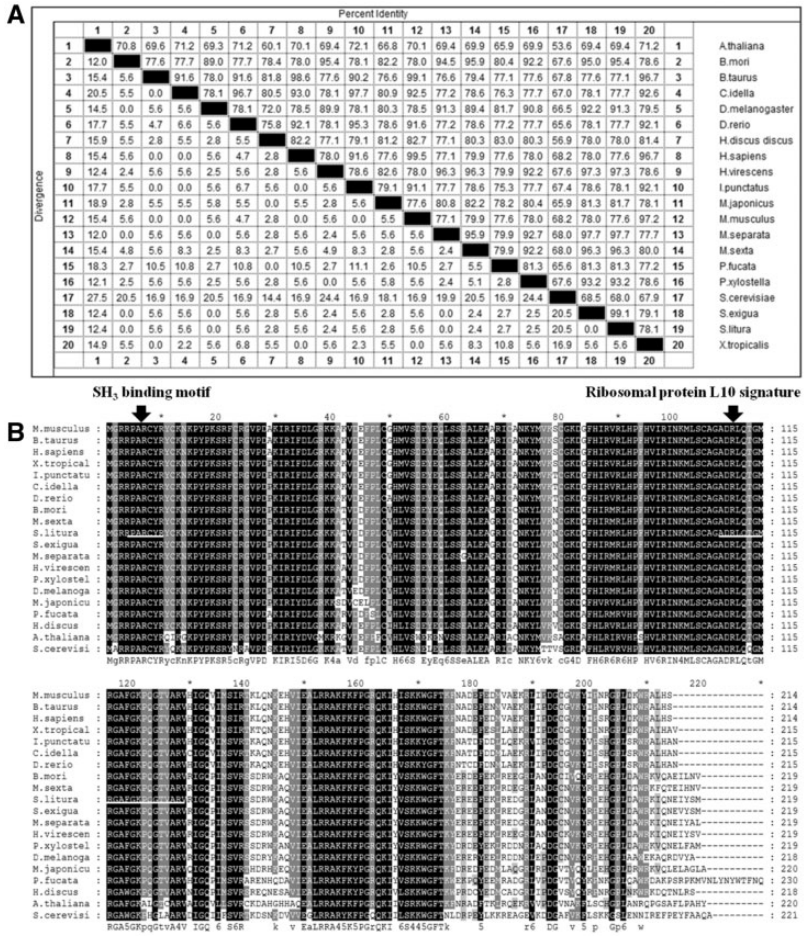

Fig. 2. The homologous analysis and multiple sequence alignment of QM family proteins. A: Matrix indicating the percentage identities of the aligned QMs. The amino acid identity percentages were calculated by the MegAlign program. B: Multiple amino acid sequence alignment of SpLQM with homologues from different species*: H. sapiens* (GenBank M64241.1)*, M. musculus* (X75312.1)*, B. taurus* (AF143815.1)*, X. tropicalis* (NP_001004965.1)*, I. punctatus* (AF401563.1)*, C. idella* (AY762997.1)*, D. rerio* (NM_200027.1)*, B. mori* (AF0 99012.1)*, M. sexta* (GU084310.1)*, S. exigua* (EU258622.1)*, M. separata* (HM467199.1)*, H. virescens* (A F368032.1)*, D. melanogaster* (U49721.1)*, M. japonicus* (EU004069.1)*, P. fucata* (AY156926.1)*, H. discus discus* (EF103442.1)*, S. cerevisiae* (X78887.1)*, A. thaliana* (CAA78856.1)*, C. sinensis* (AY641 73 3.2), and *Z. mays* (U06108). Amino acids identical in all sequences are shaded in black and similar in partial residues in gray. The characteristic domains of SpLQM are underlined in white and named on the domain.

Analysis of the SpLQM protein sequence using the PROSITE program, there were presence of several characteristic motifs, including two amidation motifs (1–4, amino acid residues ^{1}MGRR^{4}, ^{36}LGKK^{39}; Fig. 1), a cAMP- and cGMP-dependent protein kinase phosphorylation site (^{39}KKAT^{42}), two casein kinase II phosphorylation sites (^{42}TVDD^{45}, ^{174}TKYE^{177}), one N-myristoylation sites (^{114}GMRGAF^{119}), and three protein kinase C phosphorylation motifs (^{137}SVR^{139}, ^{141}SDR^{143}, ^{168}SKK^{170}). Although a ribosomal protein L10 signature (^{108}ADRLQTGMRGAFGKPQGTVARV^{129}) and an SH3-binding motif (^{4}RPARCYR^{10}) were identified in the deduced SpLQM protein, neither a signal peptide or nor a nuclear localization signal was detected.

Alignment Analysis and Phylogenetic Analysis. The QMs amino acid sequence identity and similarity percentages of different organisms were calculated using the MegAlign program. As shown in Fig. 2A, the deduced protein sequence of SpLQM shares an overall identity of 81.3–99.1 with invertebrate, 77.1–78.1% with vertebrate QM sequences, 57.4–69.4% with plants, and 68.0% with fungus and with 91.3–99.1% amino acid identity to insects. To identify the evolutionary conservation of characteristic motifs of QM proteins, multiple alignment was performed using different QM homologues (Fig. 2B). Results of Clustal W alignment indicated that the N-terminal and internal regions of SpLQM were more conserved than the C-terminal region (Fig. 2B). Moreover, it was revealed that the SH3-binding motif, ribosomal protein L10 signature were highly conserved during the evolution of QM proteins (Fig. 2B). To examine the phylogenetic relationship of SpLQM with other species, the phylogenetic tree was constructed using MEGA4.1 program by the neighbor-joining method. As shown in Fig. 3, there are four clades in the phylogenetic tree, including invertebrate (Pinctada fucata, *Haliotis discus discus, B. mori, Spodoptera exigua, Manduca sexta, Mythimna separata, Heliothis virescens, Drosophila melanogaster, Xenopus tropicalis,* and *Marsupenaeus japonicus*), vertebrate (*Danio rerio, C. idella, Ictalurus punctatus*, *Mus musculus, Bos Taurus,* and *Homo sapiens*), plants (*Arabidopsis thaliana, Camellia sinensis,* and *Zea mays*) and fungus (*S. cerevisiae*). SpLQM belongs to invertebrate (Lepidoptera) clade and has a closest genetic relationship to *S. exigua* and *M. separata.*

Genomic Structure of SpLQM. The *SpLQM* genomic DNA sequence is ~2.4 kb long and corresponds to 800 bp cDNA sequence. The exon or intron composition of the gene was determined by

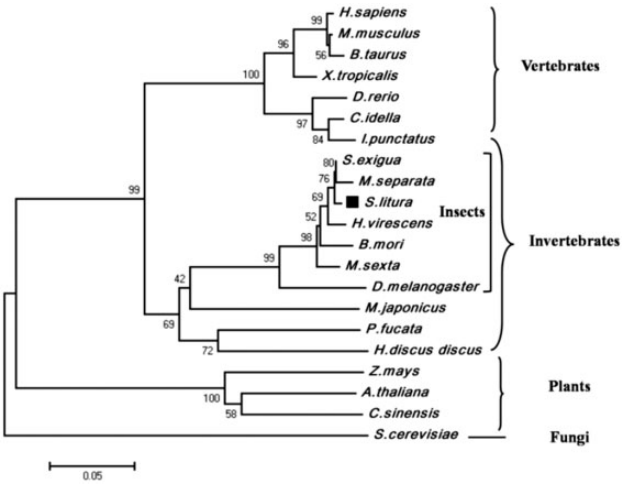

Fig. 3. Phylogenetic analysis of *SpLQM* with known QM family proteins. The tree is based on an alignment corresponding to full-length amino acid sequences, using Clustal W and MEGA (4.1, NJ). The numbers at the branches represent bootstrap values after 1,000 replications. The scale bar shows 0.05 amino acid substitutions per site.

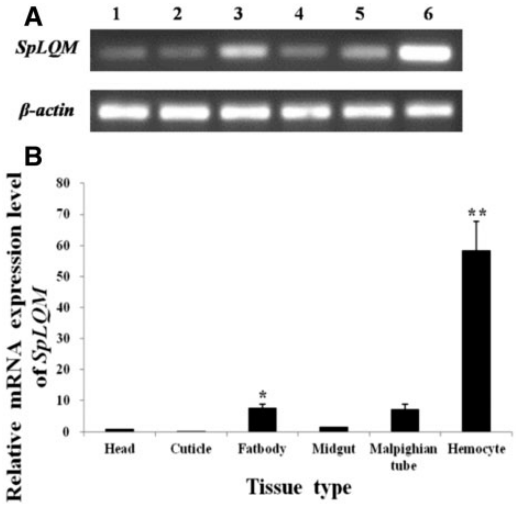

Fig. 5. Tissue distribution of *SpLQM* in *S. litura.* A. The tissue-specific expression of *SpLQM* in *S.litura* measured by semiquantitative RT-PCR. Lanes 1–6 represent head, cuticles, fatbody, midgut, Malpighian tubule, and hemocytes, respectively. *β-actin* was used as the control to normalize the amount of templates. B. The relative expression level of *SpLQM* in this tissue was further determined by qRT-PCR. The relative change in fold expression was calculated by the $2^{-\Delta\Delta CT}$ method using *β-actin* as a reference gene. The fold expression of each tissue was compared with that of heads in order to determine the tissue-specific expression. Data are presented as mean relative expression ± SEM for three replicates of real-time reactions using pooled tissues from three individual *S. litura* at each tissue. * indicates the statistically significant level at $P < 0.05$ and ** the statistically very significant level at $P < 0.01$.

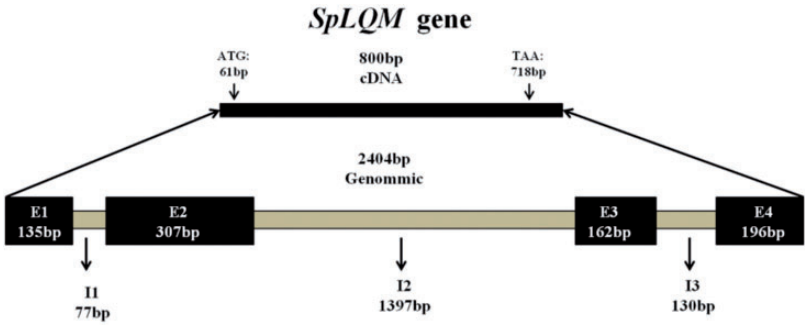

Fig. 4. Genomic organization of SpLQM. The *SpLQM* genomic sequence comprises four exons (boxes in black) and three introns (filled in gray).The positions of the start and termination codons located 61–63 of the first exon and 166–168 of the last exon in the nuclear acid sequence, respectively.

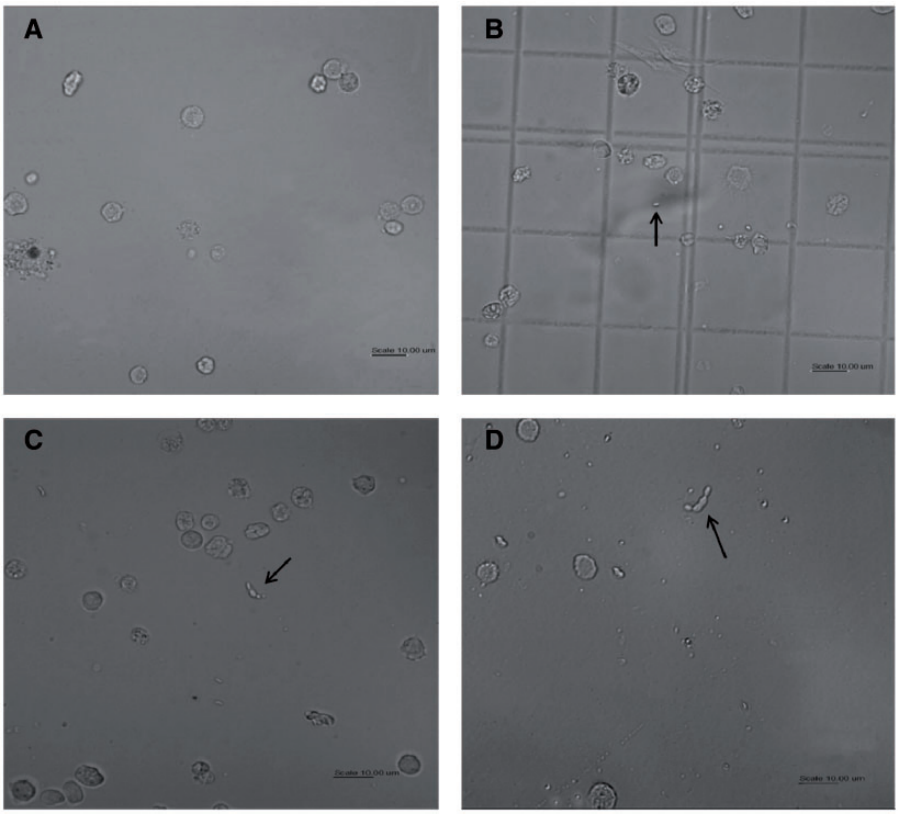

Fig. 6. The *N. rileyi* conidia proliferation in hemocoel of *S. litura.* A. The normal hemocytes collected from *S. litura* without treatment. B. The conidia and the hemocytes from *S. litura* hemocoel at 24 hpi. C. The hemocytes and the germination of spores from *S. litura* hemocoel at 48 hpi. D. The incomplete hemocytes and the rapid propagation of hyphalbody from *S. litura* hemocoel at 72 hpi. The spores are indicated by arrows.

comparing the genomic sequence with the *SpLQM* cDNA sequence. The *SpLQM* gene consists of four exons separated by three introns of different lengths (Fig. 4.) The second intron is the longest, at 1.397 kb. Exons 1–4 correspond to nucleotides 1–135, 213–519, 1,917–2,018, and 2,209–2,404 in the genomic sequence, respectively.

Tissue Expression Pattern of SpLQM. To investigate tissue distribution of *SpLQM*, total RNAs were isolated from head, cuticles, fatbody, midgut, Malpighian tubule, and hemocytes of fourth instar larvae, and subjected to RT-PCR. *SpLQM* was found to be constitutively expressed in all examined tissues (Fig. 5A). To further identify the differences in relative transcriptional level of *SpLQM* among these tissues, qPCR analysis was performed. The expression level of *SpLQM* in head was used as standard to calculate the fold change in other tissues. The results showed that the highest expression was detected in hemocytes, followed by fat bodies, while the lowest were detected in cuticles (Fig. 5B). In addition, the expression levels in fat bodies and hemocytes were much higher than that in all other tissues.

The N. rileyi Proliferation in Hemocoeleof S. litura. After inoculation with conidial suspension of *N. rileyi,* the hemolymphs collected from *S. litura* at 24, 48, and 72 h were observed with the microscope, using normal hemolymphs as control. The results showed that most spores were germinated in hemocoel of *S. litura* at 24 hpi (Fig. 6B). Then some hemocytes became incomplete at 48 hpi and simultaneously spores started to reproduce by fission (Fig. 6C). Contrast to normal control (Fig. 6A), the hemocytes were significantly declining, most even bursted, while hyphal body was rapidly reproducing at 72 hpi (Fig. 6D). Thus, *N. rileyi* may proliferate in the hemocoel.

SpLQM Transcriptional Level Affected by N. rileyi Challenge. In order to determine transcriptional response after *N. rileyi* challenge, *SpLQM* mRNA expression in fat bodies, hemocytes, and midguts of *S. litura* were determined at 0, 6, 12, 24, and 48 hpi by qPCR. The *SpLQM* in the hemocytes began to rise 6 hpi, then peaked at 12 h, and afterward gradually declined from 24 to 48 hpi (Fig. 7B); while in fat bodies, its transcript gradually went up from 6 to 12 h and peaked at 24 h post *N. rileyi* challenge (Fig. 7A). The highest induced levels of *SpLQM* transcripts in hemocytes and fat bodies were detected as 3.3-folds and 4.5-folds, respectively. Compared with the transcriptional responses in hemocytes and fat bodies, induction expression level of *SpLQM* in midguts was not as high as in hemocytes and fat bodies, but still reached 2.7-fold at 24 hpi (Fig. 7C).

Protein Expression and Characterization by SDS-PAGE and Western Blotting. SpLQM was successfully expressed in *E. coli* BL21 (DE3) with the pET-30a vector. The recombinant SpLQM expressed in *E. coli* was predominantly insoluble (not shown) and purified by gel extraction kit. This protein was further characterized by Western blotting with anti-His-Tag antibody as primary antibody (Fig. 8). Because the fusion protein had a S-tag at the C-terminus and a His-tag at both terminuses, its molecular mass was ∼32 kDa, which was higher than that of the predicted mass (Fig. 8).

Discussion

QM homologous genes were successively cloned from grass carp, shrimp, and disk abalone, and they all were demonstrated to be involved in the immune function (Yi et al. 2005, Xu et al. 2008, Oh et al. 2010). Whether is the function of lepidoptera *QM* involved in immune function remains unknown.

An EST of QM homologue was identified by examining the fatbody SSH, and then the full-length cDNA (*SpLQM*) was cloned. Analysis of the SpLQM amino acid sequence showed that it has the SH3-binding motif and a ribosomal protein L10 signature with a molecular mass of 25.6 kDa. Besides, two amidation motifs, a cAMP- and cGMP-dependent protein kinase phosphorylation site, two casein kinase II

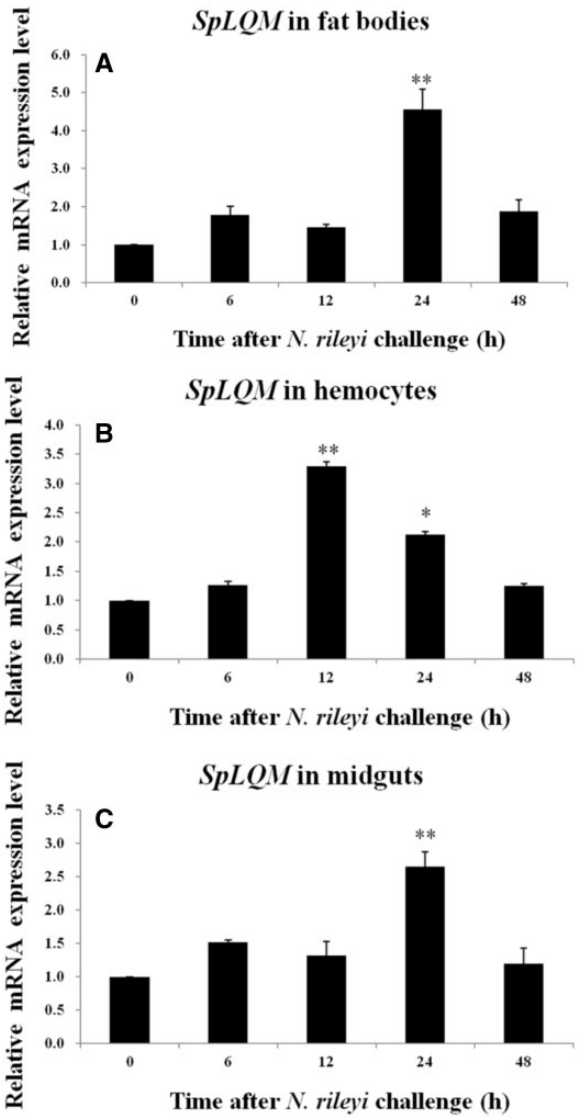

Fig. 7. Transcriptional level of *SpLQM* in different tissues after *N. rileyi* challenge. A: fat bodies, B: hemocytes, and C: midguts. Asterisks indicate significant differences ($*P < 0.05$, $**P < 0.01$) compared with that of the control (*SpLQM* mRNA at 0 hpi, control = 1). Error bars represent ± SEM of three independent PCR amplifications and quantifications.

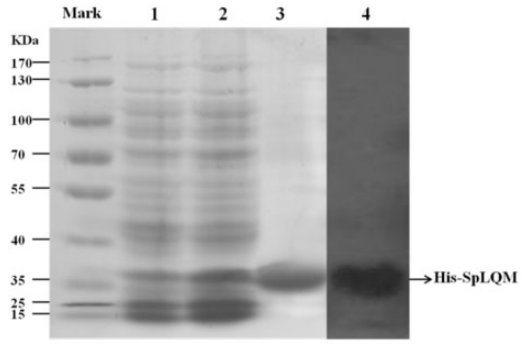

Fig. 8. SDS-PAGE and Western blotting analysis of recombinant SpLQM in *E. coli.* Mark: prestained protein Ladder; lane 1, BL21 (DE3) pLYsS transformed with pET30a only and induced by IPTG; lane 2, BL21(DE3) pLYsS transformed with pET30a-SpLQM and induced by IPTG; and lane :3, purified fusion protein SpLQM-His; lane 4, Western blotting analysis of recombinant SpLQM.

phosphorylation sites, one N-myristoylation sites, and three protein kinase C phosphorylation motifs are also found in SpLQM amino acid sequence. Most of these characteristic features perfectly match or are within the range of known QM proteins from *B. mori* (Hwang et al. 2000), shrimp PjQM (Xu et al. 2008), grass carp GcQM (Yi et al. 2005), and disk abalone AbQM (Oh et al. 2010). QM proteins are highly conserved throughout evolution. Hwang et al. (2000) found *B. mori* QM homologous has significant similarity to other insect QMs, sharing the highest degrees of sequence identify to *D. melanogaster* QM (88%). In this study, SpLQM together with *B. mori* QM is located in an insect clade, with *M. japonicus* QM in an invertebrates branch, and shares highest sequence identify to *S. exigua* (99.1%) and *M. separata* QMs (97.7%). Besides, genomic structure analysis of SpLQM showed it contains four exons and three introns, and such cases also occurred in OSQM1 from rice (Ge et al. 2002) although OSQM1protein has 64.3% sequence identify to SpLQM. The high degree of conservation suggested QMs might play critical biological roles in different species.

QM is a ribosomal proteins and participates in joining the 40S and 60S ribosomal subunits into a functional 80S ribosome; also, its incorporation into the 60S subunit is a prerequisite for the union of subunits and the initiation of translation (Eisinger et al. 1997, Loftus et al. 1997, West et al. 2005). However, be increasing evidence indicates that QM protein from various organisms has multiple extraribosomal functions sides being a constituent of the ribosome and participating in protein synthesis (Yi et al. 2005, Carvalho et al. 2008, Xu et al. 2008, Ferreyra et al. 2010, Oh et al. 2010). Previously, by screening the fatbody SSH library, we found that 117 genes upregulated by *N. rileyi* challenge, including immunity-related Gallerimycin and heat shock proteins (Chen et al. 2012). Among them, SpLQM, a homologue of a human transcript encoding the QM protein, showed increased levels after the *N. rileyi* treatments (Chen et al. 2012). In this work, we identified *SpLQM* gene in *S. litura* and found it is ubiquitously expressed in all detected tissues, with the highest mRNA level measured in hemocytes, followed by fat bodies, and the lowest in cuticles, in accordance with the expression patterns of QM in grass carp and disk abalone (Yi et al. 2005, Oh et al. 2010). Fatbody and hemocytes are the origins for the production and secretion of antimicrobial agents and activators or regulators of cellular response, while cell-mediated immunity in insects is performed by hemocytes (Hoffmann 1995; Tsakas and Marmaras et al. 2010). The higher expression of *SpLQM* in hemocytes and fatbodies suggested it might perform immune-related functions. For this reason, we selected fatbody and hemocytes tissues to analyze the transcriptional regulation of *SpLQM*. In *N. rileyi* challenge experiments, most spores did not germinate in insect hemocoel at 24 hpi (Fig. 6B), while *SpLQM* was also strongly upregulated in hemocytes and fat bodies. The expression of *SpLQM* increased faster in hemocytes than that in fat bodies; it might be closely related with hemocytes response to foreign particles first. Nevertheless, with spores rapid propagation, *SpLQM* mRNA levels started to reduce dramatically at 48 hpi in the tested tissues, which might result from the weakening insect immune response. Likewise, Yi et al. (2005) identified *QM* gene from grass carp and found it was significantly upregulated in head kidney, spleen, and liver by *Aeromonas hydrophila* and grass carp hemorrhagic virus (GCHV). In shrimp, both *PjQM* mRNA and PjQM protein were found to be significantly upregulated in white spot syndrome virus (white Spot Syndrome Virus (WSSV))-resistant shrimp by WSSV; *PjQM* was also demonstrated to regulate the activity of phenol oxidase by interaction with hemocyanin (Xu et al. 2008). In a recent study, *QM-like* gene was cloned from disk abalone and also investigated to show significant upregulation in gills upon bacterial and VHSV challenge (Oh et al. 2010). Here, we demonstrated that strongly induced expression of SpLQM by *N. rileyi* occurred in the fat bodies and hemocytes while slightly induced in midguts. The *SpLQM* expression in three tissues

could provide insights towards better understanding of the immune role QM plays against *N. rileyi* challenges.

In summary, *SpLQM* seems to be part of the initial events of the *S. litura* immune response, and its tissue-depenent and increased expression could be associated with the requirement for more functional 80S ribosomes to facilitate the production of immune-related proteins.Whether its extraribosomal functions or not was involved in *S. litura,* immune defense against *N. rileyi* infection needs to be confirmed further.These researches could contribute molecular insights into the *S. litura* immune system, but more studies are needed to elucidate the role of QM protein in innate immunity of *S. litura* in response to *N. rileyi* infection.

Acknowledgments

This work was supported by National Special Fund for Agro-Scientific Research in the Public Welfare (grant 201103002) and National High Technology Research and Development Program of China (grant 2011AA10A201).

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Received 17 October 2012; accepted 5 June 2013.