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

Yan Li, Zhong-Kang Wang, Huan Chen, Er-Yan Feng, and You-Ping Yin

Chongqing Engineering Research Center for Fungal Insecticides, School of Life Science, Chongqing University, Chongqing 400030, China

Corresponding author, E-mail: ypy128@sina.com

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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 has caused insect resistance and environmental problems (Ahmad et al. 2007). Microbial insecticides can result in a defect in protein synthesis, growth and cell division arrest, abnormalities of actin cytoskeleton, and mitochondrial respiration (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(QMs) in Saccharomyces cerevisiae protected yeast cells against oxidative stress (from H2O2, pararquat, and heat) by regulating intracellular proline levels. In Arabidopsis, QM homolog spL10 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 CQN1129 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 1×10⁸ 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 treatment 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 for 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 QMFT 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/orf/orf.f.html).

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

| Primer name                  | Primer sequence 5′–3′                  | Description                              |
|------------------------------|---------------------------------------|------------------------------------------|
| CDS III                      | ATTCTAAGGGCCAGGGCGCAGACATG-d(T)30VN   | 3′ RACE universal adaptor primer         |
| SMART II oligonucleotide     | AAGCCAGTGTATAACAGCAGAGTCGCCGG        | 3′ RACE universal adaptor primer         |
| 5′ PCR primer                | AAGCCAGTGTATAACAGCAGAGTCGCCGG        | 5′ RACE universal adaptor primer         |
| QMFP                         | TCTACGTTCACAGGAAAGGG                 | 5′ RACE universal adaptor primer         |
| QMPR                         | CTAGACGGACGTCTCTAGC                  | 3′ specific primer                       |
| QMPF1                        | GCGTGAGGACGGTCTCTAG                 | 5′ RACE specific primer                  |
| QMPR1                        | CCCATTCTGGAGAGGTAG                   | 3′ RACE nested specific primer           |
| QMFT                         | TGCTAAGAACGTGTTGTGTA                 | Full-length cDNA verification primer forward |
| QMTR                         | AGCGTGCAATCAGGGAAGG                  | Full-length cDNA verification primer reverse |
| JQMF1                        | TTTCTGGAACGGTGTTGTG                  | Genomic sequence primer                  |
| JQMR1                        | AGCAGAAACGGTGTTTAG                   | Genomic sequence primer reverse          |
| JQMF2                        | CCAATACGGCTTCTGTCCTG                 | Genomic sequence primer forward          |
| JQMR2                        | TCATGTTAATAATTTATGTTGAAAGA           | Genomic sequence primer reverse          |

Note: V = A,G,or C; N = A, G, or T.
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 \((2^{\Delta\Delta C_{t}})\) method (Livak and Schmittgen 2001).

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

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### 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   | TCTAGCTCTCC AAGAAATGG | 78                | JX134107         |
|       | DQMR   | CTGACGACCGTGTCCTCACGC |                   |                  |
|       | DACtRF | TGGACACCTCAATCTCCCCCG | 178               | DQ494753         |
|       | DACtR  | GGGCACGCCAGGAATCCAGAC |                   |                  |

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 GMGRGF (114–119), and two acylamidation sites MRGR (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.
polymerase (TaKaRa, Japan) using the primers BQMF: 5'-TTA GGATCC (BamHI) ATGGGGC GCCGGCAGCGAGATGTT-3' and BQMR: 5'-GGG AAGCTT (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 OD₆₀₀ reached 0.6, the culture was induced with isopropyl-1-thio-β-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 NaH₂PO₄, 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.
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 1MGRR4, 36LGKK39; Fig. 1), a cAMP- and cGMP-dependent protein kinase phosphorylation site (19KKAT22), two casein kinase II phosphorylation sites (42TVDD45, 174TKYE177), one N-myristoylation sites (114GMRAF119), and three protein kinase C phosphorylation motifs (137SVR139, 141SDR143, 168SKK170). Although a ribosomal protein L10 signature (108ADRLQTGMRGAFGKPQGTVARV129) and an SH3-binding motif (4RPARCYR10) were identified in the deduced SpLQM protein, neither a signal peptide 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, Camelina 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...
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 Hemocoele of 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 hemocoele 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 hemocoele.

**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
QM is a ribosomal protein 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, Lofus 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. 2005). 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

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 identity 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 identity 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 OSQM1 protein has 64.3% sequence identity to SpLQM. The high degree of conservation suggested QMs might play critical biological roles in different species.

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 (\(^{*} P < 0.05, \quad {**} P < 0.01\)) 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) pLYS5 transformed with pET30a only and induced by IPTG; lane 2, BL21(DE3) pLYS5 transformed with pET30a-SpLQM and induced by IPTG; and lane 3, purified fusion protein SpLQM-His; lane 4, Western blotting analysis of recombinant SpLQM.
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-dependent 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.

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