ISJ 19: 115-125, 2022 ISSN 1824-307X

RESEARCH REPORT

Molecular characterization and expression ananlysis of a QM protein gene from Chinese shrimp *Fenneropenaeus chinensis*

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Abstract

Based on lots of results, the QM protein was proposed to be a tumor suppressor, and facilitate in the innate immune responses, especially the prophenoloxidase (proPO) activation system. In the present study, a cDNA of 761 bp for the QM of Chinese shrimp, *Fenneropenaeus chinensis* (designated as FcQM) was cloned via rapid amplification of cDNA ends (RACE) technique. The complete cDNA sequence of FcQM contained an open reading frame (ORF) of 663 bp, 5' untranslated regions (UTR) of 42 bp and 3' UTR of 56 bp, which encoded a polypeptide of 155 amino acid residues. The analysis of the peptide sequence of FcQM revealed that FcQM sequence contained a Ribosomal_L16 domain, an SH3-binding motif, an N-acylation site, two putative antibiotic binding sites, two putative protein kinase C phosphorylation sites and two acylamidation sites, which shared over 80 % similarity with previously identified QM protein genes. Quantitative real-time PCR (qRT-PCR) results showed that the transcript of FcQM was extensively distributed in all the tested tissues and most highly expressed in hemocytes and hepatopancreas. After challenged with *Vibrio anguillarum* or white spot syndrome virus (WSSV), the FcQM transcripts were significantly increased ($p < 0.05$) both in hepatopancreas and hemocytes. Hence, the analysis of sequence and transcription suggested that FcQM might play crucial roles responses to bacterial and viral invasion in innate immunity of arthropods. This study is the first report of the QM protein gene from *F. chinensis*, which would enrich the understanding of the innate immune defense mechanism in shrimp.

Key Words: *Fenneropenaeus chinensis*; innate immunity; QM protein gene

Introduction

Chinese shrimp, *Fenneropenaeus chinensis*, is one of the most important mariculture species in China. It is mainly distributed along the coast of Yellow Sea and Bohai Sea and the west coast of Korean Peninsula (Meng *et al*., 2021). In recent years, outbreaks of diseases caused by bacteria, such as *Vibrio parahaemolyticus*, and viruses, such as white spot syndrome virus (WSSV) have resulted in mass shrimp mortality, seriously inhibiting the production and economic income of the shrimp industry nationwide (Liu *et al*., 2005; Wang *et al*., 2013). The invasion of pathogens drives the fluctuations of physiological and behavioral status of shrimp, which could be harmful to the survival of shrimps (Alfaro *et al*., 2021; He *et al*., 2022). Due to the lack of adaptive immune system, shrimps only rely on innate immune system to protect the organism from pathogens (Destoumieux-Garzon *et al*., 2001). Therefore, it is particularly important to better understand the innate immune defenses mechanism to promote the development of disease treatment, and then to find more effective ways to solve the problems.

The QM protein gene was originally identified from human in the non-tumorigenic Wlims' cell line G401 using subtractive hybridization method (Dowdy *et al*., 1991). Initially, it was found that the QM protein gene was expressed at a higher level in non-tumorigenic Wlims' cells than in the

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tumorigenic parental cells, speculating that the QM protein gene might be a tumor suppressor gene (Chen *et al*., 2012). In addition, The QM protein gene encodes the ribosomal protein L10, which is required for the formation of the 80S ribosome (Loftus *et al*., 1997). Because of its presumed functions, the QM protein gene has been the subject of intensive study. Homologues of QM have been identified in various species, include vertebrates and invertebrates and have been shown to play important immune roles in cell growth, tissue differentiation and apoptosis (Green *et al*., 2000). The mouse QM protein gene is differentially expressed throughout the embryo, and the expression pattern of QM suggests that QM might be involved in the posttranslational protein processing, playing an important role in the differentiation of specific tissues during embryogenesis (Mills *et al*., 1999). The homolog of QM in chicken (JIF-1), a negative transcription regulator of c-Jun, could bind to the protooncogene c-Jun and suppressed AP-1 function, suggesting that QM protein gene may be involved in transcriptional regulation (Imafuku *et al*., 1999). And in *Bombyx mori*, QM protein gene interacted with Jun protein and negatively regulated the expression of AP-1, which could affect some immune genes expression (Zhou *et al*., 2019). Accumulating evidence has demonstrated that QM protein are involved in regulating the immune response of organisms. In grass carp, QM protein was significantly up-regulated by *Aeromonas hydrophila* and grass carp hemorrhagic virus (GCHV) infection, suggesting that QM protein gene is associated with anti-pathogens inflammatory response (Wen *et al*., 2005). Following bacterial stimulation, QM protein gene transcription was significantly increased in the hepatopancreas of clams, demonstrating that QM transcriptional expression can be induced by pathogens challenge and play an active role in immune responses (Cui *et al*., 2019). As one of the major invertebrate immune responses, prophenoloxidase activation system (proPO-AS) plays an important role in innate immunity (Soderhall *et al*., 1994). Through pathogens-associated molecular patterns (PAMPs) such as lipopolysaccharide (LPS), peptidoglycan (PG) or β-1,3-glucan (BG), bind to specific shrimp humoral pattern recognition proteins (PRPs) and activate the proPO system (Sritunyalucksana *et al*., 1999; Sritunyalucksana *et al*., 2000; Cerenius *et al*., 2004). The QM protein in large yellow croaker could regulate the activity of phenol oxidase (PO) (Han *et al*., 2015). The QM protein in Penaeus japonicus can interact with hemocyanin and myosin, thereby regulating the activity of PO in the innate immunogen activation system of arthropods (Xu *et al*., 2008). In shrimps, QM protein can significantly improve bacterial clearance and positively regulate PO activity (Liu *et al*., 2014). More interestingly, researchers reported for the first time that the QM protein was present in crustaceans as a potential peptidoglycan recognition protein (PGRP) in *Penaeus monodon* and activated the proPO response (Udompetcharaporn *et al*., 2014). Therefore, QM protein may play an important role in the innate immune response of shrimp.

In this study, we cloned the full-length cDNA of the FcQM gene and analyzed the sequence features and molecular characteristics of the FcQM protein. Subsequently, the prawns were challenged with *V. anguillarum* and white spot syndrome virus (WSSV) to explore the tissue-specific expression of the FcQM gene. Finally, we analyze the immune response of QM protein gene to pathogens challenges.

Table 1 Oligonucleotide primers used in the present study

Primer	Sequence (5'-3')	Tm (°C)	Brief information
adaptor primer	GGCCACGCGTCGACTAGTAC	60	Anchor primer for 3' and 5' RACE
adaptor primer-oligo (dT)	GGCCACGCGTCGACTAGTACT_{17}VN	-	Oligo (dT) for cDNA synthetize
adaptor primer-oligo (dG)	GGCCACGCGTCGACTAGTACG_{10}HN	-	Oligo (dT) for cDNA synthetize
Fc-18S-qRT-F	TATACGCTAGTGGAGCTGGAA	58	Internal control for real-time PCR
Fc-18S-qRT-R	GGGGAGGTAGTGACGAAAAAT	57	Internal control for real-time PCR
FcQM-CDS-F	ATGGGGCGCCGTCCGGCCCGATGC	77	Gene specific primer for CDS
FcQM-CDS-R	TTAAGCAAGACCAGCAAGTTCCAACTG	64	Gene specific primer for CDS
FcQM-qRT-F	GGCTTCACCAAGTTCGACC	58	Gene specific primer for real-time PCR
FcQM-qRT-R	TTAAGCAAGACCAGCAAGTTCC	59	Gene specific primer for real-time PCR
FcQM-RACE-F1	TCATTGAGGCTCTCCGACGTGCCA	68	Gene specific primer for 3' RACE
FcQM-RACE-F2	TGGTCGCCTTAGGCCTGATGGTGT	67	Gene specific primer for 3' RACE
FcQM-RACE-R1	CCTAAGTCATAGATACGAATCTTG	54	Gene specific primer for 5' RACE
FcQM-RACE-R2	TTACAGTAACGGTAGCATCGGGCCGGA	69	Gene specific primer for 5' RACE
M13-47	CGCCAGGGTTTTCCCAGTCACGAC	56	Vector primer for sequencing
RV-M	GAGCGGATAACAATTTCACACAGG	56	Vector primer for sequencing

Materials and methods

Experimental shrimps, immune challenge and sample collection

In the present study, the shrimps weighing 8 - 12 g were obtained from a local shrimp farm in Qingdao, China, and cultured in the laboratory for two weeks before processing. In this phase, Shrimps were cultured in 640 L cylindrical tanks with 500 L air-pumped circulating seawater at 20 ± 1 °C. To study the tissues-specific expression of FcQM, the hemolymph and tissues, including eyestalk, gill, gonad, heart, hepatopancreas, intestine (mid gut), muscle, nerve and stomach were collected from at least fifteen untreated shrimps. To determine the immune response of FcQM, approximately 360 shrimps were employed for microbe stimulation assay. To determine the immune response of FcQM, approximately 360 shrimps were employed for microbe stimulation assay. The shrimps were randomly divided into three groups. The shrimps of control groups were received challenges at the abdominal segment with 100 µl phosphate buffered saline (PBS, pH 7.4, 10010023, Thermo Fisher Scientific, USA). The shrimps of two treatment groups *V. anguillarum* suspension (1×10^4 CFUs μl^{-1}, in PBS) and WSSV stock (1×10^4 copies μl^{-1}, in PBS), respectively. The *V. anguillarum* suspension and WSSV stock were prepared according to previous reports (Yi *et al*., 2014; Xia *et al*., 2015; Sha *et al*., 2016). Hepatopancreas and hemolymph samples were removed from shrimps of the bacteria-injected, WSSV-injected and the control groups at 0, 3, 6, 12, 24, 36 and 48 hpi. The shrimp hemolymph was collected from the ventral sinus into a sterilized syringe with an equal volume of modified anticoagulant Alsever solution (trisodium citrate 30 mM, NaCl 510 mM, citric acid 200 mM, glucose 115 mM, and EDTA 10 mM, pH 7.4), and centrifuged at 800 × g for 10 min at 4 °C. All of the tissue samples were kept in RNAlater (AM7020, Thermo Fisher Scientific, USA), which snap-frozen in liquid nitrogen and used for RNA isolation.

Total RNA isolation and cDNA synthesis

The total RNA was extracted from hemolymph and tissues, including eyestalk, gill, gonad, heart, hepatopancreas, intestine (mid gut), muscle, nerve and stomach, using the TRIzol Reagent (Takara, Dalian, China) following the manufacturer's protocol. The RNA concentration was determined by measured UV absorbance at 260 nm with a Nanodrop Lite. Its completeness was examined by electrophoresis on a 1.5 % agarose gel. According to the manufacturer's instructions, the synthesis of first-strand cDNA was carried out with Promega M-MLV (M1701, Promega, USA). Briefly, a mixture containing 1 µg of total RNA, 2 µL of adaptor primer-oligo (dT) or random primers and 5 µL of RNase Free ddH_2O was incubated at 70 °C for 5 min. Then, 4 µL of 5 × M-MLV buffer, 2 µL of DTT (100mM), 1 µL of dNTP (10mM), 1 µL of ribonuclease inhibitor, 1 µL of M-MLV and RNase Free ddH_2O were added (total 20 µL). The mixture was incubated at 42 °C for 1 h and terminated at 95 °C for 5 min before cooling on ice. Then, the synthesized cDNA was stored at 80 °C for further use.

Molecular cloning of FcQM cDNA sequence

The partial length sequence of FcQM cDNA was obtained from the shrimp transcriptome sequencing database (Genbank accession number: BM302396) (Zhang *et al*., 2010). Two pairs of sequence-specific primers, FcQM-RACE-F1/F2 and FcQM-RACE-R1/R2 (Table 1), were designed using Primer Premier 5.0. The sequence-specific primers and adaptor primers were used to clone the full-length cDNA of FcQM by 5' and 3' RACE techniques. Specific primers FcQM-RACE-F1, FcQM-RACE-F2 and adaptor primer-oligo(dT) were employed to obtain 3' ends sequence of FcQM using a semi-nested PCR. Then, specific primers FcQM-RACE-R1, FcQM-RACE-R2 and adaptor primer-oligo(dG) were employed to obtain 5' ends sequence of FcQM using a semi-nested PCR. And the coding sequence (CDS) of FcQM was amplified and confirmed using another two gene-specific primers, FcQM-CDS-F/R (Table 1). Amplification was performed in 25 µL reaction volume, containing 2.5 µL of 10 × PCR buffer, 1.5 µL of $MgCl_2$ (25 mM), 2.0 µL of dNTP (2.5 mM), 1.0 µL of each primer (10 mM), 1.0 µL of template, 0.2 µL of Ex Taq (TaKaRa, Japan) and 15.8 µL of RNase Free ddH_2O. The protocol of PCR was one initial denaturation cycle 94 °C for 5 min, followed by 35 cycles of 94 °C for 10 s, 55 °C for 30 s and 72 °C for 30 s, 5 min at 72 °C for the final extension. All PCR amplification were performed in a VeritiPro Thermal Cycler (ThermoFisher, USA). The PCR products were gel-purified and then cloned into the pMD-18T Simple (Takara, Japan). After being transformed into the competent cells Escherichia coli strain DH5α (Tiangen, China). The positive recombinants were identified through anti-ampicillin selection and verified by PCR screening using vector primers, M13-47 and RV-M (Table 1). The positive recombinants ware carried out using an ABI 3730 sequencer (ThermoFisher, USA).

Bioinformatics analysis of FcQM cDNA and protein sequences

The FcQM nucleotide sequence was translated into the amino acid sequence using the DANMAN 8.08 software package. The FcQM amino acid sequence was used for protein functional domains prediction and analysis using the Simple Modular Architecture Research Tool (SMART, smart.embl-heidelberg.de). And the calculated molecular mass and theoretical isoelectric point (pI) were predicted using the ExPASy Molecular Biology Server (web.expasy.org/compute_pi). Similar QM sequences of other species were searched in NCBI gene database using the BLAST program. And multiple alignments were performed using ClustalW2. Finally, an unrooted phylogenetic tree was constructed using amino acid neighbor-joining (NJ) method with MEGA 10.0 software and tested for reliability with 1000 bootstrap replications.

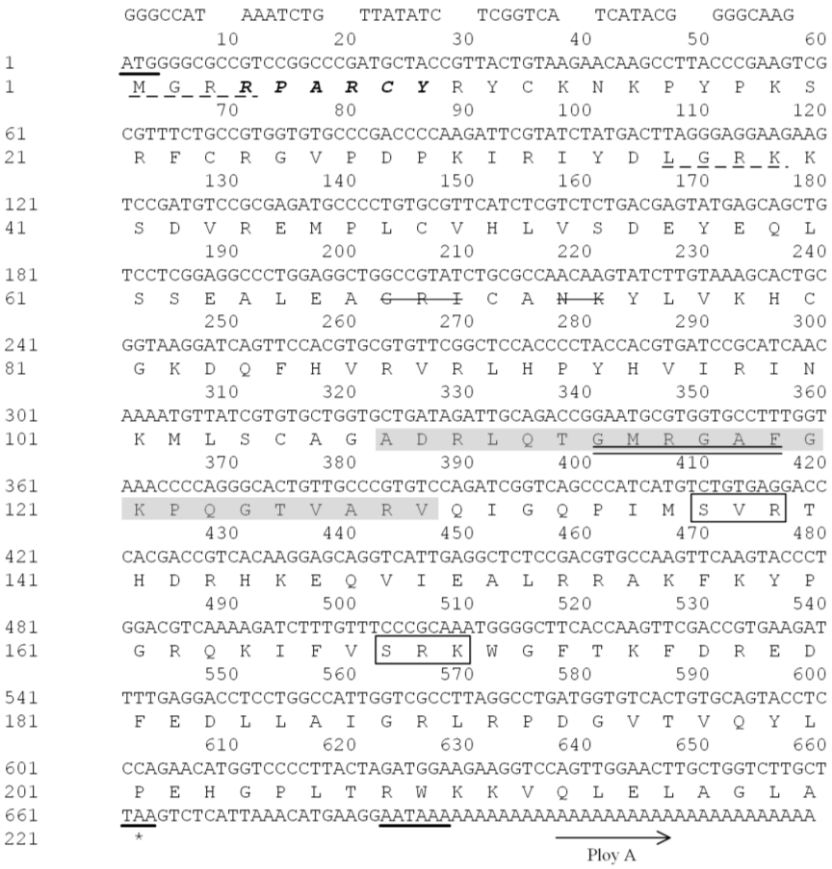

Fig. 1 Nucleotide and deduced amino acid sequences of FcQM (GenBank Accession KU361824). The start codon (ATG), the stop codon (TAA), and the polyadenylation signal sequence (ATTAAA) are in bold and underlined. The predicted SH3-binding motif (RPARCY) are in bold italics, two acylamidation sites (MGRR) and (LGRK) are underlined with dotted lines, two putative antibiotic binding sites (GRI) and (NK) are shaded with a line, an N-acylation site (GMRGAF) are double-underlined and two protein kinase C phosphorylation sites (SVR) and (SRK) are boxed. In addition, a ribosomal protein L10 signature is in shaded

Quantitative Real-time PCR analysis of FcQM mRNA expression

The transcripts of FcQM in various tissues from healthy *F. chinensis* and its temporal expression in hemocytes and hepatopancreas after *V. anguillarum* or WSSV challenges were detected by qRT-PCR. Two FcQM gene-specific primers, FcQM-qRT-F and FcQM-qRT-R (Table 1), were designed using Primer Premier 5.0 to detect the temporal expression of the FcQM gene. 18S rDNA was chosen as reference gene for internal standardization. Two primers, Fc-18S-qRT-F and Fc-18S-qRT-R (Table 1) were used to amplify the fragments of 18S rDNA. qRT-PCR was carried out in a 20 μL reaction volume containing 10 μL of 2 × ChamQ SYBR qPCR Master Mix (Q311-02, Vazyme, China), 0.4 μL of each primer (10 mM), 2 μL of the 1:10 diluted cDNA and 7.2 μL of RNase Free ddH_2O. The protocol of qRT-PCR was one initial denaturation cycle 95 °C for 30 s, followed by 39 cycles of 95 °C for 10 s and 60 °C for 30 s. After each run, a dissociation step (95 °C for 15 s, 60 °C for 60 s, 95 °C for 15 s) was performed to generate a melting curve thermal profile to verify a single product. All qRT-PCR amplification were performed in an ABI 7300 (ThermoFisher, USA). The mRNA relative expression level of FcQM and 18s rDNA were analyzed by comparative CT method ($2^{-\Delta\Delta Ct}$ method) (Schmittgen *et al*., 2008; Wang *et al*., 2011). All data were given in terms of the mRNA relative expression of FcQM as the mean ± standard deviation and subjected to one-way ANOVA followed by Duncan's multiple range test by using IBM SPSS Statistics 23.0.0.0. The P values less than 0.05 were considered statistically significant.

Results

Cloning and characterization of FcQM cDNA

To further characterize the functions of QM protein gene in shrimp immune response against pathogens infections, QM protein gene was cloned from *F. chinensis*. The full-length cDNA (Genbank accession number: KU361824) of FcQM was obtained by 5' and 3' RACE techniques. As is shown in Fig. 1, it comprised 761bp, containing an open reading frame (ORF) of 663 bp, 5'

untranslated regions (UTR) of 42 bp and 3' UTR of 56 bp. The ORF encoded a 155 amino acid polypeptide with a calculated molecular mass of approximately 25.53 kDa and a theoretical isoelectric point (pI) of 10.08. SMART indicated that FcQM contains a Ribosomal_L16 domain (from C12 to F166), an SH3-binding motif (RPAFCY), an N-acylation site (GMRGAF), two putative antibiotic binding sites (GRI; NK), two putative protein kinase C phosphorylation sites (SVR; SRK), two acylamidation sites (MGRR; LGRK). In addition, it contains a ribosomal L10 signature motif. A polyadenylation signal (AATAAA) was found upstream of PolyA.

Homology and phylogenetic analysis of FcQM

Homology and similarity analysis of the deduced amino acid of FcQM was performed by GeneDoc and DNAMAN software to determine the evolutionary conservation of characteristic motifs and active sites of QM proteins. The amino acid sequence homology of the QM protein of *F. chinensis* and *P. vannamei* was the highest, and the amino acid sequence homology of the QM protein of *Homo sapiens* was the lowest. In addition, the amino acid sequence of FcQM was similar to that of *Homo sapiens* (75 %), *Penaeus japonicus* (92 %), *Eriocheir sinensis* (92 %), *Danio rerio* (77 %), *Pinctada fucata* (78 %), *Drosophila melanogaster* (79 %), *Bombix mori* (80 %), and *Pediculus humanus corporis* (81 %) had higher homology in Fig. 2. The alignment results show that the N-terminal and internal region of the peptides are conserved. In addition, the study also found that SH3-binding motif (RPARCY), ribosomal protein L10 signature (ADRLQTGMRGAWGKQGTVARV) and protein kinase C phosphorylation site (RPARCY) were highly conserved during the evolution of QM protein.

In order to study the molecular evolution of FcQM genes, a neighbor-joining (NJ) phylogenetic tree based on the amino acid sequences of 16 different species was constructed to analyze the phylogenetic relationship of FcQM. As is shown in Fig. 3, it was clearly to find that the QM group can be divided into mammalians (*O. aries*, *B. taurus*, *H. sapiens*, *M. musculus*), amphibians (*X. tropicalis*), fish (*D. rerio*, *L.crocea, O. niloticus*) , mollusks (*H. diversicolor supertexta*, *H. discus discus*, *P. fucata*, *A. farreri, M. meretrix*), arthropods (*P. vannamei*, *F. chinensis*, *P. japonicus*, *P. clarkii*, *D. melanogaster, P. monodon, B. mandarina*) four groups. Further analysis showed that FcQM is the closest relative to LvQM and is located in the crustacean subclade.

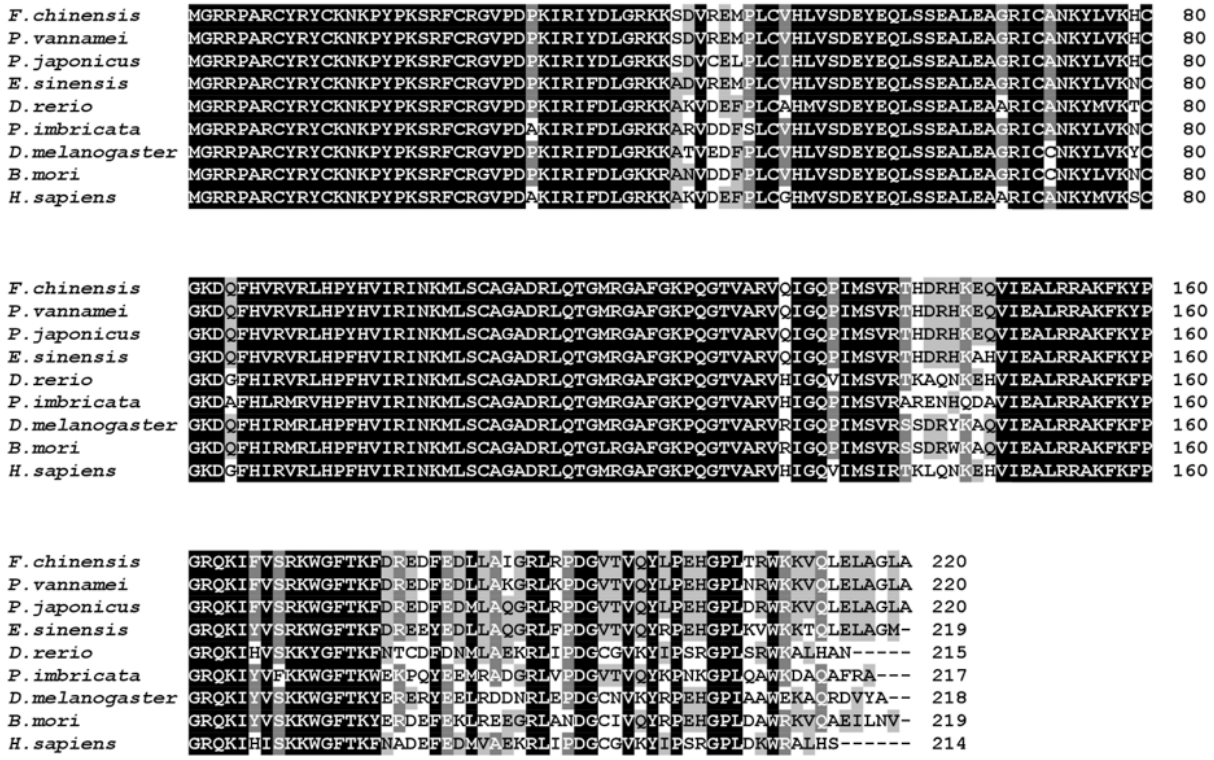

Fig. 2 Multiple alignments of FcQM with previous known QM. The sequences and their accession numbers are as follows: *Homo sapiens*, AAB27665.1; *Fenneropenaeus chinensis*, KU361824; *Penaeus vannamei*, AGA16579.1; *Penaeus japonicus*, ABS45569.1; *Eriocheir sinensis*, ATO74510.1; *Danio rerio*, AAV34163.1; *Pinctada fucata*, AAN85578.1; *Drosophila melanogaster*, AAC16108.1; *Bombyx mori*, AAK73358.1

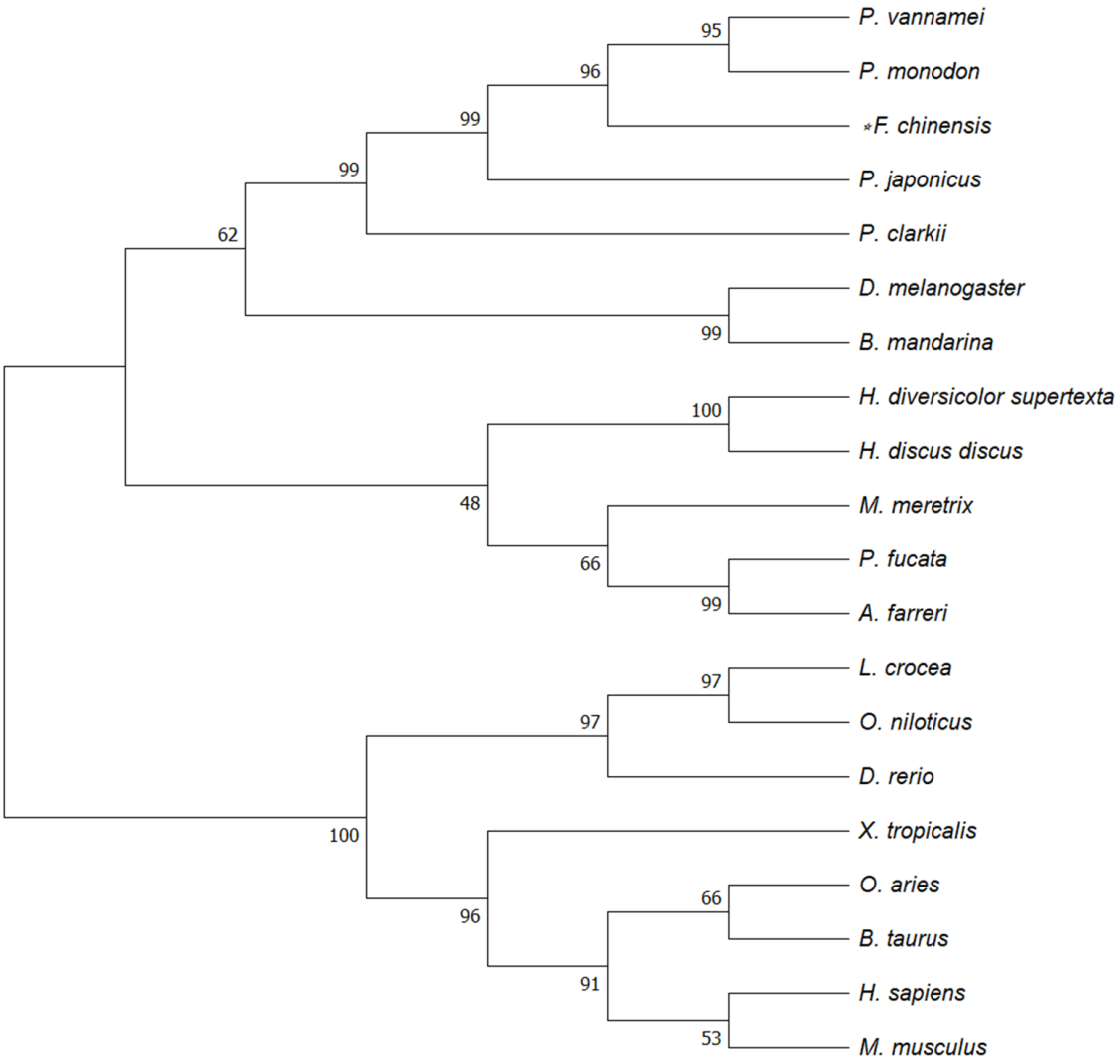

Fig. 3 Consensus neighbor-joining phylogenetic based on the protein sequences of FcQM from different organisms. The evolutionary history was inferred using the Neighbor-Joining method. The bootstrap consensus tree inferred from 1,000 replicates was taken to represent the evolutionary history of the taxa analyzed. All positions containing gaps and missing data were eliminated. The numbers at the forks indicated the bootstrap value. The sequences and their accession numbers are as follows: *Penaeus vannamei*, AGA16579.1; *Fenneropenaeus chinensis*, KU361824; *Penaeus japonicus*, ABS45569.1; *Procambarus clarkii*, AEB54638.1; *Drosophila melanogaster*, AAC16108.1; *Haliotis diversicolor supertexta*, ACJ71721.1; *Haliotis discus discus*, ABO26700.1; *Pinctada fucata*, AAN85578.1; *Azumapecten farreri*, AKM12718.1; *Danio rerio*, AAV34163.1; *Larimichthys crocea*, ACS93602.1; *Xenopus tropicalis*, NP_001004965.1; *Ovis aries*, ABV64839.1; *Bos taurus*, NP_777185.1; *Homo sapiens*, AAB27665.1; *Mus musculus*, CAA53061.1; *Meretrix meretrix*, ANG56314.1; *Oreochromis niloticus*, XP_003454286.1; *Bombyx mandarina*, AAC98301.1; *Penaeus monodon*, ACV72062.1

Tissue-specific expression of FcQM

The relative expression level of FcQM in various tissues including eyestalk, gill, gonad, heart, hemocytes, hepatopancreas, intestine, muscle, nerve and stomach was detected by qRT-PCR with 18S rDNA as the internal control in fifteen untreated shrimps. As shown in Fig. 4, FcQM genes were all expressed in the tested tissues, with the highest expression in hepatopancreas, higher expression in hemocytes, and less expressed gills, eye stalk, gonad, heart, intestine, muscle, nerve, stomach expression.

The mRNA expression profiles of FcQM post bacterial stimulation

In order to understand the changes in the transcriptional response of FcQM gene after stimulation by *V. anguillarum*, qRT-PCR was used to detect the mRNA relative expressions of FcQM in hemocytes and hepatopancreas at various time points (0, 3, 6, 12, 24, 36, 48 hpi). As shown in Fig. 5, the relative expression level of FcQM in both tissues was up-regulated under the *V. anguillarum* stimulation, while the expression level of FcQM in the control group had no significant change. Fig. 5A

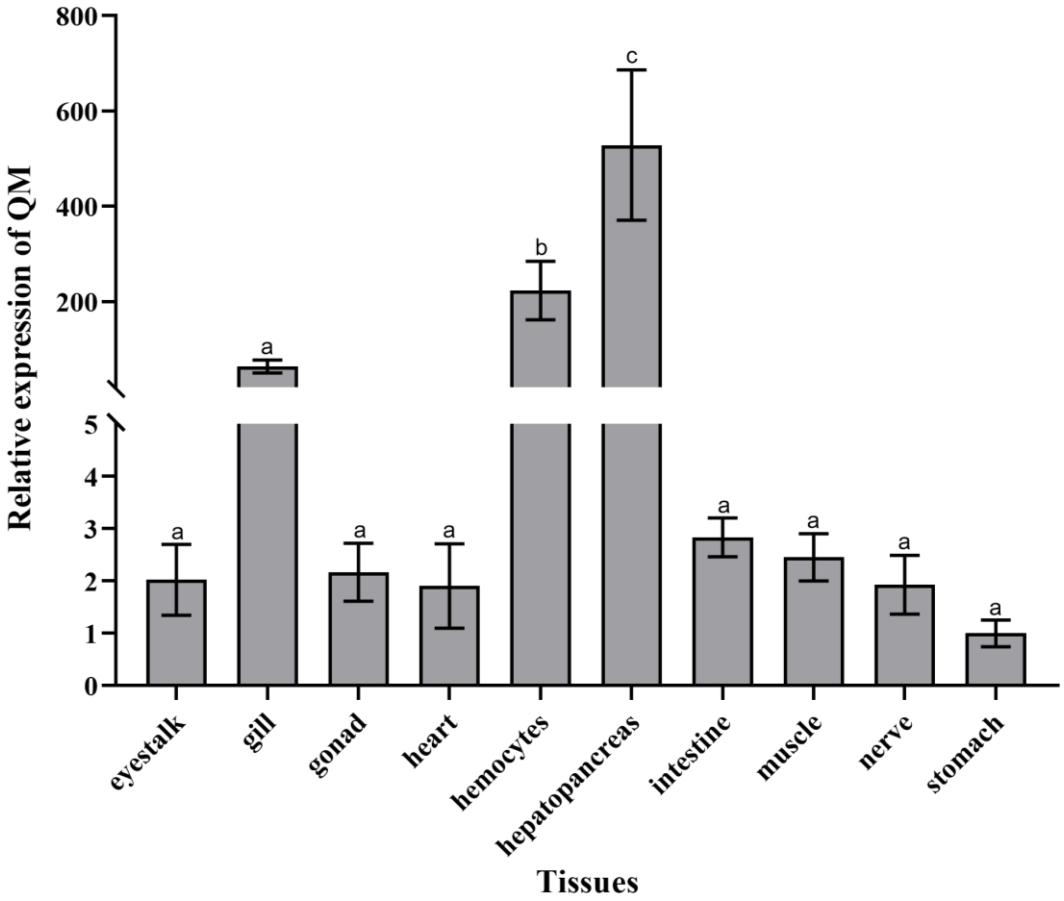

Fig. 4 Tissue distribution of FcQM mRNA transcripts detected by qRT-PCR technique. The 18s rDNA gene was used as an internal control to calibrate the cDNA template for each sample. Vertical bars represent mean ± SD (n = 3), and bars with different characters were significantly different ($p < 0.05$), while bars with same characters were not significantly different

shows that the relative expression of FcQM gene in hemocytes after *V. anguillarum* stimulation was significantly increased at 3 hpi and 6 hpi ($p < 0.05$) with the peak value of 6.41-fold higher than that in the control group at 3 hpi. The expression level of FcQM gene in the treatment group were not significantly different from that in the control group from 12 hpi to 48 hpi. Fig. 5 B shows that the relative expression of FcQM gene in the hepatopancreas after immune stimulation. The expression level of FcQM were significantly increased at 12 hpi and 24 hpi after *V. anguillarum* challenges ($p < 0.05$) and reached a peak value at 24 hpi with the peak value of 7.87-fold higher than that in the control group, and recovered to 36 hpi at 36 hpi. normal level. The expression level of FcQM gene did not exhibit any significant changes in control group.

The mRNA expression profiles of and FcQM post WSSV stimulation

Under WSSV stimulation, the expression of FcQM in hemocytes and hepatopancreas was up-regulated. As shown in Fig. 6 A, the relative expression of FcQM in hemocytes after WSSV challenges was significantly up-regulated at 3 hpi and 6 hpi ($p < 0.05$) with the peak value of 6.58-fold higher than that in the control group at 6 hpi. After 6 hpi, the expression level of FcQM was the same as that of the control group. There was no significant difference, and gradually returned to normal level. Fig. 6 B shows that under WSSV stimulation, FcQM also showed a higher expression level. The highest expression level (2.9-fold) of FcQM transcripts was detected at 12 hpi after the challenge with WSSV ($p < 0.05$). In addition, the expression level of FcQM gene was fluctuated at 48 hpi, representing 2.49-fold higher level than that in the control group.

Discussion

Since the first QM protein gene was identified from humans, the QM protein gene has been identified in a large range of organisms. Recent research achievements indicated QM protein may play an important role in the innate immune defenses mechanism of shrimp (Wen *et al*., 2005). In the present study, we first reported the identification and molecular characterization of

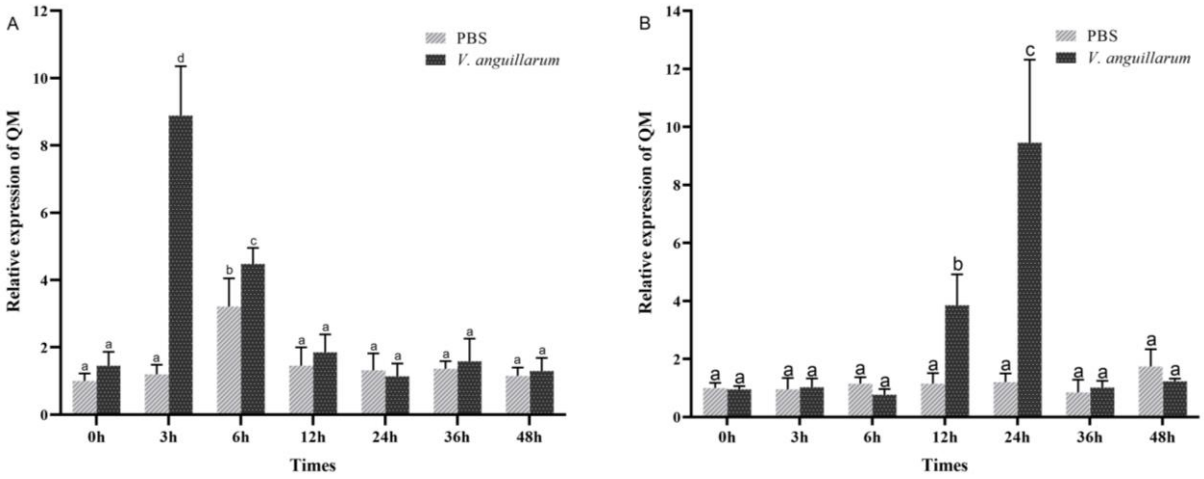

Fig. 5 Temporal mRNA expression profiles of FcQM detected via qRT-PCR technique in shrimp hemocytes and hepatopancreas at 0, 3, 6, 12, 24, 36 and 48 hpi stimulation. A: Temporal mRNA expression profiles of FcQM post *V. anguillarum* stimulation in shrimp hemocytes; B: Temporal mRNA expression profiles of FcQM post *V. anguillarum* stimulation in shrimp hepatopancreas; The 18s rDNA gene was used as an internal control to calibrate the cDNA template for each sample. Vertical bars represent mean ± SD (n = 3), and bars with different characters were significantly different ($p < 0.05$), while bars with same characters were not significantly different

FcQM cDNA sequence from the Chinese shrimp, *F. chinensis*. The full-length cDNA of FcQM was 761bp, including a 663 bp ORF encoding a 155 amino acids polypeptide with a calculated molecular mass of approximately 25.53 kDa and a pI of 10.08. The protein sequence of FcQM shared over 80 % similarities with other identified QMs. The deduced amino acid sequence of FcQM was conformity to the conserved domains of those identified QMs of marine invertebrates, which included an SH3-binding motif, an N-acylation site, antibiotic binding sites, protein kinase C phosphorylation sites, acylamidation sites and a ribosomal L10 signature motif, indicating that FcQM was an orthologue of QM family (Liu *et al*., 2014; Han *et al*., 2015; Cui *et al*., 2019). The highly conserved signature sequences might suggest the important role of QM in the basic functions. Phylogenetic analysis showed that no signal peptide identified from the deduced amino acid sequence of FcQM, pointed out that FcQM may not be a secretory protein. A study confirmed that QM localized to the cytoplasmic face of the rough ER on mammalian cells and yeast (Loftus *et al*., 1997). In the NJ phylogenetic tree, FcQM is the closest relative to LvQM and is located in the crustacean subclade, which could have similar functions to those from other invertebrates. The sequence characteristics and the phylogenetic relationship of FcQM indicate that the FcQM gene could have similar functions to those from other invertebrates.

Previous research showed that QM was ubiquitously expressed in various tissues. Interestingly, it was also reported that the QM protein gene participated in the innate immune response. In order to better understand the potential biological role of QM in shrimp, the tissue distribution of FcQM mRNA transcripts was detected by qRT-PCR technique. The FcQM mRNA transcripts were observed to be ubiquitously expressed in all the examined tissues, including eyestalk, gill, gonad, heart, hemocytes, hepatopancreas, intestine, muscle, nerve and stomach, which were consisted with the previously observation in other invertebrates (Xu *et al*., 2008; Li *et al*., 2016; Cui *et al*., 2019). The ubiquity of FcQM mRNA transcripts indicated that it could be involved in many physiological responses of shrimps. The highest expression level of FcQM mRNA was found in hepatopancreas and hemocytes, followed by gill. Similarly, in *M. meretrix*, MmQM was highest expressed in hepatopancreas and hemocytes, followed by gill (Cui *et al*., 2019). The hepatopancreas was considered as the main immunity and detoxification organ (Song *et al*., 2015). Hemocytes as the major immune cells respond to pathogens mainly through phagocytosis (Jiravanichpaisal *et al*., 2006). Gill is part of the mucosal system, functioning as the first defense line against pathogens in lower animals (Wang *et al*., 2019). Therefore, the high expression level of FcQM in these tissues further confirmed that FcQM could participate in the innate immune system of shrimp.

Accumulating evidence has shown that QM protein genes have important immune functions in organisms. Previous research have shown that LvQM mRNA transcripts in hemocytes and hepatopancreas increased significantly in the early stage of the experiment after *V. anguillarum* stimulation, and RNAi and bacterial clearance experiments showed that QM protein regulated the activity of PO (Liu *et al*., 2014). To further investigate the role of FcQM in immune responses, the present research detected the temporal expression profiles of QM in response to a bacterial

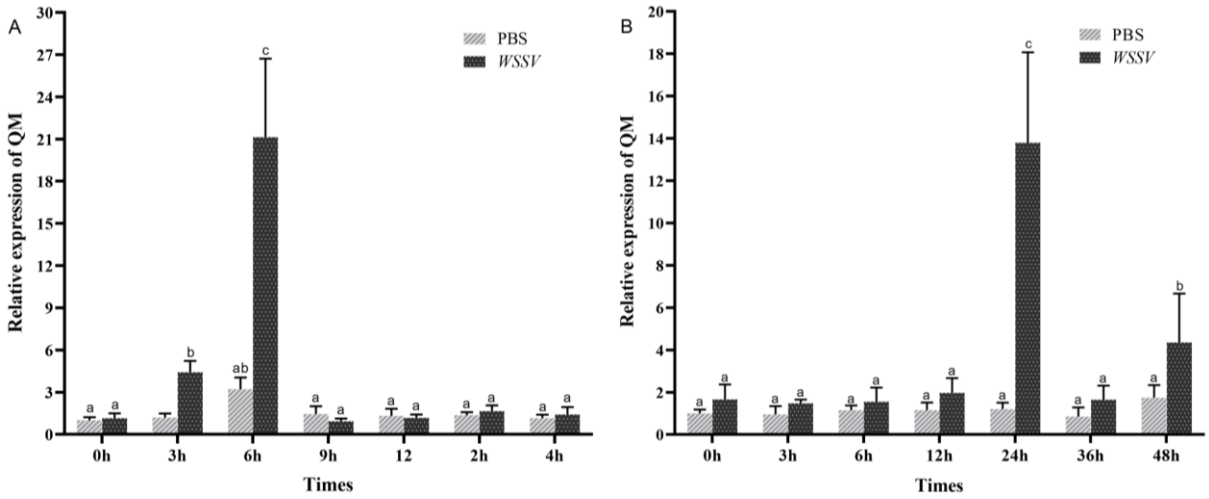

Fig. 6 Temporal mRNA expression profiles of FcQM detected via qRT-PCR technique in shrimp hemocytes and hepatopancreas at 0, 3, 6, 12, 24, 36 and 48 hpi stimulation. A: Temporal mRNA expression profiles of FcQM post WSSV stimulation in shrimp hemocytes; B: Temporal mRNA expression profiles of FcQM post WSSV stimulation in shrimp hepatopancreas; The 18s rDNA gene was used as an internal control to calibrate the cDNA template for each sample. Vertical bars represent mean ± SD (n = 3), and bars with different characters were significantly different ($p < 0.05$), while bars with same characters were not significantly different

and WSSV challenge. The expression level of FcQM gene after *V. anguillarum* and WSSV stimulation was significantly increased at 3 hpi and 6 hpi in hemocytes and increased at 12 hpi and 24 hpi in hepatopancreas, which suggest the important immune regulation effect of FcQM in *F. chinensis*. The expression of FcQM in hemocytes is earlier than that in hepatopancreas. Gill is the first defense line against pathogens in shrimps. However, once this barrier is passed, a series of complex innate humoral and cellular immune reactions is induced in both haemocoel and tissues, resulting in a fast elimination of pathogens (Cerenius *et al*., 2010). This also reasonably explains that the differential expression level of FcQM in hemocytes is earlier than that in hepatopancreas in this study. In hemocytes, the induction expression level of FcQM mRNA transcripts reached a peak value at 3 hpi with 6.41-fold after *V. anguillarum* stimulation and at 6 hpi with 6.58-fold after WSSV stimulation. In hepatopancreas, the induction expression level of FcQM mRNA transcripts reached a peak value at 24 hpi with 6.41-fold and 6.58-fold after *V. anguillarum* or WSSV stimulation. Researchers have reported that the results of qRT-PCR and Western blot indicated that the PjQM gene was significantly upregulated in WSSV-resistant *Penaeus Japonicus* (Xu *et al*., 2008). In the *Chlamys farreri*, the relative expression of CfQM sharply increased at 6 hpi and normalized at 48 hpi under *V. anguillarum* and acute viral necrobiotic virus stimulation, which suggested that CfQM can protect organisms against pathogens challenges (Chen *et al*., 2015). Bacterial challenge tests indicated that HdiQM gene expression was induced by the bacterial isolates from *Haliotis diversicolor*, which implied that HdiQM might be an inflammatory stress-inducible gene associated with pathogen infection (Li *et al*., 2016). The above results further verify that QM may play an essential role in the response to pathogens. Therefore, QM protein gene have important immune functions in shrimps and other invertebrates, especially in process of innate immune defense. Specially, the upregulation of the expression level of FcQM at 48 h after WSSV stimulation, which is different from the other results, suggests that FcQM might be involved in multiple innate immune response processes in shrimp.

In summary, we have successfully cloned the cDNA sequence of FcQM from the Chinese shrimp, *F. chinensis*. Tissue expression analysis indicated that FcQM was detected in all examined tissues, and higher expressed in hemocytes, hepatopancreas and gills. The FcQM was significantly increased both in hemocytes and hepatopancreas after *V. anguillarum* and WSSV stimulation. These results of sequence and transcription analysis suggested that FcQM may play crucial roles in innate immune responses to bacterial and viral stimulation.

Acknowledgments

This research was supported by the Project of Sanya Yazhou Bay Science and Technology City Management Foundation (SKJC-KJ-2019KY01), and the Startup Fund of Young Talents Project of Ocean University of China. We would like to thank the expert reviewers for constructive suggestions and enlightening comments during the revision.

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