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Short sequence report

# Molecular characterization and immune response expression of the *QM* gene from the scallop *Chlamys farreri*

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ABSTRACT

The scallop *Chlamys farreri* is an important aquaculture species in northern China. However, the sustainable development of the scallop industry is currently threatened by several pathogens that cause mass mortality of this mollusk. Therefore, a complete understanding of the immune response mechanisms involved in host–virus interactions is necessary. This study reports a novel *QM* gene from *C. farreri*. This gene was first identified as a putative tumor suppressor gene from human and then confirmed to participate in several functions, including immune response. The *QM* gene from *C. farreri* (CfQM) was identified by suppression subtractive hybridization, and its full-length (763 bp) cDNA was obtained through rapid amplification of cDNA ends. The cDNA of CfQM contained a short 5′-UTR of 22 bp and a 3′-UTR of 84 bp. Its ORF comprised 657 nucleotides that encode 218 amino acids with a molecular weight of approximately 28.3 kDa and an isoelectric point of 10.06. The deduced amino acid sequence of CfQM contained a series of conserved functional motifs that belong to the QM family. Phylogenetic analysis revealed that CfQM was closely related to other mollusk QM proteins, and altogether they form a mollusk QM protein subfamily that displays evolutionary conservation from yeast to human. The tissue-specific expression and transcriptional regulation of CfQM were investigated by quantitative real-time PCR in response to bacterial (*Vibrio anguillarum*) and viral (acute viral necrobiotic virus) challenges. The transcript level of CfQM was high in all of the examined tissues in a constitutive manner. The highest and lowest expression levels of CfQM were measured in the hepatopancreas and hemocyte, respectively. Upon bacterial and viral challenges, the relative mRNA expression of CfQM sharply increased at 6 h post-infection (hpi) and then normalized at 48 hpi. These findings suggest that CfQM can respond to and protect against pathogen challenge. To the best of our knowledge, this study is the first report of the *QM* gene from scallop. The results presented herein provided new insights into the molecular basis of host–pathogen interactions in *C. farreri*.



## 1. Introduction

The Zhikong scallop *Chlamys farreri* is an important native species of China. In the last decades, this bivalve mollusk has been widely cultured in the coastal provinces of northern China. In addition, the *C. farreri* industry has been an important part of marine aquaculture in the country. However, the frequent occurrence of mass mortality has posed a great threat to the sustainable development of this industry in recent years. This phenomenon can be ascribed to a combination of several factors, including temperature, salinity, pathogen, and overcrowded culture density [1,2], among which pathogen infection is the most important. *Vibrio* is a genus of pathogenic bacteria that are ubiquitous among aquaculture animals in seawater; *Vibrio* infection is characterized by several symptoms, such as loose adductor muscle, mucid mantle, atrophied or tumefacient hepatopancreas, decreased feeding rate, and even halted feeding [3]. Acute viral necrosis virus (AVNV) [4] is primarily responsible for up to 90% of the cumulative mass mortality of *C. farreri* within 2 or 3 d [4].

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New insights into the innate immune mechanisms of *C. farreri* against foreign pathogens may facilitate to identify potential biomarkers and develop anti-pathogen approaches. Therefore, many studies have focused on this topic in recent years [3,5–7] and several genes have been discovered to be involved in the immune response of *C. farreri* against foreign pathogens, including C-type lectin [8], TNF receptor-associated factor 6 [9], lipopolysaccharide and β-1, 3-glucan binding protein [10], peptidoglycan recognition protein [11], and allograft inflammatory factor-1 [5].

The novel gene QM was originally identified from human through subtractive hybridization as an elevated cDNA transcript in a nontumorigenic Wilm's tumor microcell hybrid relative to the tumorigenic parental cell line [12]. The *QM* gene was originally associated with tumor suppression because of its high expression level in non-tumorigenic Wilm's tumor microcell hybrid [12]. Considerable attention has been paid to this gene in various species because of its important putative function. To date, the *QM* gene has been identified and cloned from a wide range of living organisms, including vertebrates such as mammals [12] and fish (*Ctenopharyngodon idellus*) [13], invertebrates such as *Drosophila melanogaster* [14] and *Marsupenaeus japonicus* [15], yeast (*Saccharomyces cerevisiae*) [16], and even fungi [13]. Moreover, the deduced amino acid sequences of QMs were highly conserved throughout the eukaryotic evolution, indicating that QM has fundamental and vital functions across species. Substantial evidence indicated that QM is involved in various life processes, including signal transduction [13], translation and transcription [17,18], membrane-associated protein synthesis [19], cell growth, differentiation, and apoptosis [20–22]. In particular, recent studies have shown that QM plays an important role in immune response. The QM expression level of grass carp (*C. idellus*) challenged by pathogens (*Aeromonas hydrophila* and grass carp hemorrhagic virus) could be significantly upregulated, which suggests that QM is an inflammatory stress-inducible gene associated with anti-bacterial and viral defense [13]. Similarly, disk abalone (*Haliotis discus discus*) displayed an upregulated expression level of the *QM* gene in response to bacterial (*Vibrio alginolyticus*, *Vibrio parahemolyticus*, and *Listeria monocytogenes*) and viral (viral hemorrhagic septicemia virus) challenges [23]. A recent study has demonstrated that the *QM* gene from pacific white shrimp (*Litopenaeus vannamei*) was associated with its host defense against *Vibrio anguillarum* infection [24].

The *QM* gene has been identified from various organisms; however, only a few marine animals, including *H. discus discus* [23] and *L. vannamei* [24], and a few mollusks, including *Pinctada fucata* [25], *Pinctada martensii* [23], and *Haliotis diversicolor supertexta* [23], have appeared to have this gene. The present study reports a novel *QM* gene (CfQM) from *C. farreri*. The full-length cDNA of CfQM was obtained through rapid amplification of cDNA ends (RACE). Then, the tissue-specific expression of the *QM* gene was investigated. Finally, the immune response of the gene to bacterial and viral challenges was analyzed. To the best of our knowledge, this study is the first to identify and characterize *QM* gene from scallop.

## 2. Materials and methods

### 2.1. Scallops, immune challenge, and sample collection

Healthy scallops (*C. farreri*) with an average shell height of 52.3 ± 0.2 cm and an average wet weight of 13.23 ± 1.15 g were purchased from an aquatic product market located at Weihai, Shandong Province, China. The scallops were acclimated in several 25 L tanks supplied with 10 L of aerated and filtered seawater at 25 °C. The following conditions were maintained throughout the experiment: salinity, 30‰; oxygen content, > 6 mg/L; and pH, 7.5 to 8.1.

Various tissues (50 mg), including gill, hepatopancreas, muscle, and mantle, were dissected from healthy scallops to analyze the tissue-specific expression of CfQM. Particularly, hemolymph was withdrawn from the adductor muscle sinus of scallops with a 21-gauge needle attached to a 1 mL sterilized syringe. The hemocyte mixture was then centrifuged for 10 min at 800 g at 4 °C to collect hemocyte pellet. The samples from three individual scallops were combined as an analytical sample, and four replicates were performed for each type of tissue.

For viral challenge, viral suspension (VS) and control supernatant were respectively prepared from AVNV-infected and healthy *C. farreri* in accordance with the methods described by Chen et al. [26]. After 5 d of acclimatization in the laboratory, the live scallops were equally divided into two groups (infection and control groups) and then randomly stocked into four 25 L tanks. The diluted AVNV solution (VS $5^{-1}$) at an infection dose of 50 μL per scallop was injected in the adductor muscle of the scallops in the infection group. This infection dose was adopted from a previous experiment [26] to guarantee the success of infection and gain live infected scallops from which representative hemocyte samples could be isolated for RNA extraction. Similarly, the scallops in the control group were also injected in the adductor muscle with the control supernatant (50 μL per individual) at a 5-fold dilution. For bacterial challenge, *V. anguillarum* was incubated on 2216E medium at 28 °C for 18 h and then used to prepare a bacterial suspension with phosphate buffered saline (PBS) at a final density of $1 \times 10^7$ CFU/μL. Similar to the AVNV infection experiment, the scallops were acclimated for 5 d and then injected with 50 μL of bacterial suspension or PBS. Hemolymph samples were randomly obtained from the scallops in both infected and control groups at 0, 3, 6, 12, 24, and 48 h post infection (hpi). The hemolymphs from four individual scallops were combined as one analytical sample, and four replicates were performed for each time point. The mixtures were then centrifuged at 800 g for 10 min at 4 °C to collect hemocyte pellets. All of the hemocyte samples were snap-frozen in liquid nitrogen and used for RNA isolation.

### 2.2. Suppression subtractive hybridization (SSH) cDNA library construction and expressed sequence tag (EST) analysis

A forward SSH cDNA library representing the up-regulated genes of *C. farreri* against AVNV infection was constructed with hemocyte mRNA from the AVNV-infected and normal scallops in our previous study [27]. Random sequencing of 288 positive colonies from the library with T3 primer yielded 275 successful sequencing reactions. BLAST analysis revealed that an EST of 624 bp was homologous to the previously identified QM protein of *Crassostrea ariakensis* (Accession no. ACO07302) with a similarity of 89% and an *E*-value of $1e^{-105}$. Therefore, this EST sequence was used to further clone the full-length cDNA of QM from *C. farreri*.

### 2.3. RNA isolation and cDNA synthesis

Total RNA was extracted from pooled samples by using RNAiso Plus (Takara, Dalian, China) according to the manufacturer's protocol. The extracted total RNA was dissolved in RNase-free water. The concentration and purity (A260/280) of the isolated nucleic acid was determined using a NuDrop Micro-Spectrophotometer (NAS99, ACTGene, USA), and its integrity was examined through 1% agarose gel electrophoresis. RNA samples were stored at −70 °C until use. cDNA was synthesized using the PrimeScript® RT reagent kit with gDNA Eraser (TaKaRa, Dalian, China) in accordance with the manufacturer's instructions. In brief, a 10 μL mixture containing