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Cloning and expression analysis of mitogen-activated protein kinase (MAPK) p38 in pearl oyster *Pinctada fucata martensii*

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Abstract: The mitogen-activated protein kinase (MAPK) signaling pathway is crucial in cellular response to extracellular stimuli. This pathway utilizes serine/threonine-protein kinases that transmit extracellular signals through a phosphorylation cascade to cells. Rapid-amplification of cDNA ends (RACE) was utilized for cloning and quantitative PCR (qPCR) used for expression profiling of p38 MAPK in this study. Our findings reveal that the *Pinctada fucata martensii* (*PmMAPK p*38) has a full-length cDNA of 1 516 bp, an open reading frame (ORF) of 1 071 bp, and has an estimated molecular mass of 40.88 ku which is encoded 356 amino acids. Domain prediction analysis indicates that *PmMAPK p*38 has the typical MAPK family S_TKc domain and sequence alignment, tree construction, and MatGAT calculation demonstrate its high similarity and conservation to MAPK genes in other species. Our qPCR results show that *PmMAPK p*38 is extensively expressed in various *P. fucata martensii* tissues, with the highest levels in hepatopancreas, followed by mantle, and the lowest levels in adductor muscle. Stimulation with LPS resulted in relative expression peaking at 2 h, decreasing to the least at 12 h. The greatest expression was roughly 5 times higher than the lowest. After stimulation with *Vibrio harveyi*, relative expression peaked at 2 h, decreased to the lowest at 8 h, with the highest expression approximately 4 times greater than the lowest. Our findings suggest that *PmMAPK p*38 may be a crucial component of the immune response in *P. fucata martensii*, and this study provides essential data for further investigation on the immune defense system of shellfish.

Key words: *Pinctada fucata martensii*; mitogen-activated protein kinase (MAPK) p38; immunity; gene cloning

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Pinctada fucata martensii is the primary species used in China and Japan for marine pearl aquaculture [1]. "South China Sea Pearls", which constitute more than 95% of all Chinese seawater pearls, are

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renowned for their production from *P. fucata martensii*. The production of pearls involves grafting a mantle cut from a donor oyster and seeding a nucleus into the host oyster's gonad. This process is known to trigger the immune response in *P. fucata martensii* which can be fatal [2].

The immune system of *P. fucata martensii* relies on cell-mediated and humoral reactions to eliminate naturally occurring marine pathogens [3]. Due to their less evolved status and the lack of adaptive immunity, mollusks mainly rely on the recognition of invaded cells and phagocytosis for immune function [4]. The vascular system in *P. fucata martensii* is open, and its non-specific immunity significantly relies on hemolymph [5]. This process involves identifing alien invasions, known as recognition of infection, and activating serine proteases or deactivating protease inhibitors. The infection signal is amplified for a more dangerous signal, eventually stimulating signaling pathways that alter gene transcription [6]. The effector transcription system is activated to produce immune effects such as antimicrobial peptides, phenoloxidase, or apoptotic system members [7].

MAPK is a signaling component that translates extracellular stimuli into a wide variety of cellular responses [8]. Eukaryotic cells contain multiple MAPK pathways that regulate a vast range of cellular activities including gene expression, mitosis, metabolism, motility, survival, apoptosis, and differentiation [9]. A conserved tertiary kinase pattern constitutes the basic component of the MAPK pathway from yeast to humans. The MAP kinases family among mammals consists of three groups: ERKs, JNKs, and p38/SAPKs[10]. Various extracellular signals stimulate MAPK pathways, which result in different cellular responses. Research has shown that MAPK pathways can intertwine with other ligand-induced signaling pathways, leading to orchestrate a complex set of cellular events that ultimately determine cellular response [11]. The activation of *MAPK p*38 was triggered when *Littorina littorea* cells were exposed to a suspension of phorbol ester (PMA), lipopolysaccharide (LPS), and mannan [12]. MAPK p38 is a subclass of MAPKs and functions as a stress-activated protein kinase. At present, five isomers of MAPK p38, namely p38α (p38), p38β1, p38β2, p38γ, and p38δ, have been identified. These isomers may exhibit different responses to the same stimulus.

Even though *MAPK p*38 has been extensively researched in other species, its study has been limited in *P. fucata martensii*. This study determined the full-length cDNA sequence of *PmMAPK p*38 via rapid-amplification of cDNA ends (RACE). mRNA expression in the hemolymph of *P. fucata martensii* was evaluated using quantitative polymerase chain reaction (qPCR) after two different infections. The results of this analysis can be used as a foundation for further research into the relationship between immunity and nuclear transplantation.

1 MATERIAL AND METHODS

1.1 Animals and sample collection

Ninety *P. fucata martensii* pearl oysters, approximately 2 year old with a shell length of 5-6 cm were collected from the Xuwen seawater pearl cultivation base, Zhanjiang, Guangdong Province, China. The animals were cultured in tanks of circulating seawater at 25-27 °C for one week before the experiments. During this period, healthy animals with similar sizes were carefully selected for the follow-up experiments. The study 's animal experimental procedures were carried out according to the "Animal Experimentation Regulations of Guangdong Ocean University". The operators strictly followed the experimental protocols during the experiment.

1.2 RNA extraction

Total RNA was extracted from *P. fucata martensii* tissues including mantle edge (ME), hepatopancreas (HP), adductor muscle (A), gill (GI), and hemolymph (HE) using TRIzol according to the manufacturer 's guidelines (Invitrogen, USA). The RNA concentration and purity were immediately detected using a NanoDrop 2000 spectrophotometer (Thermo-Fisher Scientific USA) and agarose gel (1%) electrophoresis, respectively. Extracted RNA with

A_{260}/A_{280} = l.8-2.0 was stored at −80 °C ultra-low temperature freezer for future use.

1.3 Cloning of *PmMAPK p*38 cDNA

The SMARTer™ RACE cDNA amplification kit (Clontech, USA) was used for cloning the full-length *PmMAPK p*38 cDNA. The cDNA clone was stored in a refrigerator at −20 °C.

The target gene was amplified using the RACE technique. Special primers were used to screened the *P. fucata martensii* hemocyte transcriptome for unigene fragment of *P. fucata martensii* MAPK [2]. The primers used in the experiment are presented in Table 1. A positive fragment was obtained in two rounds of PCR for 5′ and 3′ RACE PCR. The first-round PCR was performed on a cDNA template prepared using the universal primer (UPM) and the 5′ outer primer. The first-round PCR products were then used as templates for nested PCR with primers 5′ inner and universal primer (NUP). The PCR was consisted as follows: an initial step of denaturation at 94 °C for 5 min followed by 30 cycles of denaturation at 94 °C for 30 s, annealing at 63 °C for 30 s, and extension at 72 °C for 3 min. The PCR was completed by a final extension at 72 °C for 10 min. The 3′-end was amplified in the same. The PCR products were analyzed using 1% agarose gel electrophoresis and purified using a PCR purification kit (Promega, USA). The purified PCR product was ligated into a pMD18-T vector (TaKaRa, Japan) and then transformed into competent *Escherichia coli* cells. The resulting products were sequenced by the DNA Sequencing Service of Sangon (Shanghai, China).

1.4 DNA sequencing and bioinformatics analysis

We firstly obtained *PmMAPK p*38 nucleotide sequences, and then performed bioinformatics analysis. DNAMAN 6.0 was used to splice nucleotide sequences. The open reading frames (ORFs) were analyzed using ORF Finder (http://www.ncbi.nlm.nih.gov/gorf/gorf.htmL). The protein domains were predicted via SMART online (http://smart.embl-heidelberg.de/[13]). Nucleotide sequences were translated into the amino acid sequence using the Rimer software. Signa IP 4.1 Server was used to predict signal peptides (http://www.cbs.dtu.dk/services/SignalP/[14]). The protein functional sites were predicted via Softberry (http://linux1.softberry.com/[15]). ExPASy was used to predict molecular weights and isoelectric points (http://web.expasy.org/protparam/[16]), and online software sets (http://distillf.ucd.ie/porterpaleale/[17]) was used separately to analyze the tertiary structure and hydrophobicity of proteins. TMHMM Server V2.0 was used to predict the presence or absence of transmembrane regions (http://www.cbs.dtu.dk/services/TMHMM/[18]). SWISS-MODEL was used to predict protein 3D structure (https://swissmodel.expasy.org/interactive[19]).

Tab. 1 Nucleotide primers used in the cloning of *PmMAPK p*38

primers	sequences (5′-3′)	application
3′ inner	GAAGATGCTGGATTTGGATGCCGACAC	inner PCR
3′ outer	TTGCTTGAAAAGATCAACAGCCCTGAGG	outer PCR
5′ inner	CGTATCCCGTCATCTCATCCTCCGTGT	inner PCR
5′ outer	CCTCAGGGCTGTTGATCTTTTCAAGCAA	outer PCR
NUP	AAGCAGTGGTATCAACGCAGAGT	inner PCR
UPM-long	CTAATACGACTCACTATAGGGCAAGCAGTGGTATCAACGCAGAGT	outer PCR
UPM-short	CTAATACGACTCACTATAGGGC	outer PCR
GAPDH-F	GCAGATGGTGCCGAGTATGT	qRT-PCR
GAPDH-R	CGTTGATTATCTTGGCGAGTG	qRT-PCR
PmMAPK-F	CCGTTTGGGAAGTGTCTCA	qRT-PCR
PmMAPK-R	TAAGGTTCTGCCGTGGGT	qRT-PCR
M13-F	CGCCAGGGTTTTCCCAGTCACGAC	colony PCR
M13-R	AGCGGATAACAATTTCACACAGGA	colony PCR

The DNAMAN 6.0 software was used to perform multiple sequence alignment and calculated sequence identity and the similarity among all sequences with MatGAT 2.0. [20] MEGA 7.0 [21] was used to construct the biological evolutionary tree.

1.5 Infection experiments

Ninety healthy *P. fucata martensii* were randomly divided into three groups: lipopolysaccharides (LPS), *Vibrio harveyi*, and phosphate buffered saline (PBS). The LPS challenge group was intramuscularly injected with 100 μL (10 μg/mL) of LPS (*E. coli*, O55∶B5, Sigma) in PBS, the bacterial challenge group was intramuscularly injected with 100 μL of *V. harveyi* resuspended in PBS with the concentration of 10^7 cells/mL, while the control group was injected with 100 μL of PBS. Six time points (0, 2, 4, 8, 12, and 24 h; 0 as the control group) were selected. Haemolymph from three individuals was pooled to represent a single replicate in order to minimize individual variability. Three replicates were used for each time point. The haemolymph was collected from the adductor muscle using a syringe, and the haemocytes were harvested by centrifuging at 800×*g*, 4 °C for 10 min. The haemocyte pellets were immediately subjected to RNA extraction using TRIzol reagent (Invitrogen, USA).

1.6 Quantitative real-time PCR (qRT-PCR) assay and statistical analysis

Five tissues (hepatopancreas, mantle, gill, hemocytes, and adductor muscle) were selected as controls and were not subjected to any infections. Hemolymph was extracted *P. fucata martensii* at different time points for RNA isolation. The RNA was then reverse-transcribed into cDNA samples, which was amplified using specific primers for qPCR analysis. The quantity of the *PmMAPK p*38 mRNA was measured using the $2^{-\Delta\Delta C_t}$ method[22]. The relative expression level of *PmMAPK p*38 mRNA was normalized to that of the housekeeping gene, glyceraldehyde-3-phosphate dehydrogenase (GAPDH). One-way analysis of variance (ANOVA) was used to detect significant differences in *PmMAPK p*38 mRNA expression levels between the experimental and control groups at each time point using SPSS (v.19.0; IBM, USA). $P<0.05$ were considered significant difference.

2 RESULTS

2.1 *PmMAPK p*38 cDNA cloning and bioinformatics analyses

The full-length cDNA of *PmMAPK p*38 consisted of 1 516 bp, with a 1 071 bp open reading frame, 5' untranslated region (5' UTR) of 126 bp, and 3' untranslated region (3' UTR) of 445 bp (GenBank: MN378562.1). The open reading frame encodes a precursor with 356 amino acids (Fig.1). The protein was estimated to have a molecular mass of 40.88 ku, and the theoretical isoelectric point of 5.91.

The lowercase letters indicate 5′ UTR and 3′ UTR and the capital letters indicate open reading frame (ORF); the MAPK signatures and conserved "MEEVD" motif are shadowed; the ATP binding domain and canonical polyadenylation signal sequence are underlined; the conserved "GxxGxG" motif is marked in bold letters and has been italicized.

The sequence of *PmMAPK p*38 exhibited significant similarity to other Bivalves' *MAPK p*38 (Fig. 2). The amino acid sequence of *PmMAPK p*38 possesses one N-glycosylation site, five protein kinase C (PKC) phosphorylation sites, six casein kinase II phosphorylation sites, three N-cardamom acylation sites, one CAAX box, three microbody C-terminal localization signal sequences, and two specific protein kinase ATP-binding regions. *PmMAPK p*38 lacks a transmembrane region, classifying it as a non-transmembrane protein. Signal peptide prediction software revealed that the N-terminal of the MAPK polypeptide lacks a signal peptide, indicating that this protein is non-secretory. Online software was use to predict the secondary structure of MAPK, and the results showed that the protein contained 52.53% alpha-helix, 6.74% beta-sheets 12.08% extension chain, and 28.65% irregular curl. Domain analysis revealed a serine/threonine-protein kinase (S_TKc) (Pfam:CL0016; Fig.3) domain and a highly conserved motif composed of Thr-Gly-Tyr (TGY).

Using SWISS-MODEL, we constructed the 3D structure of PmMAPK p38 protein, which exhibits structural similarity to MAPK p38 (PDB:3fkl) of

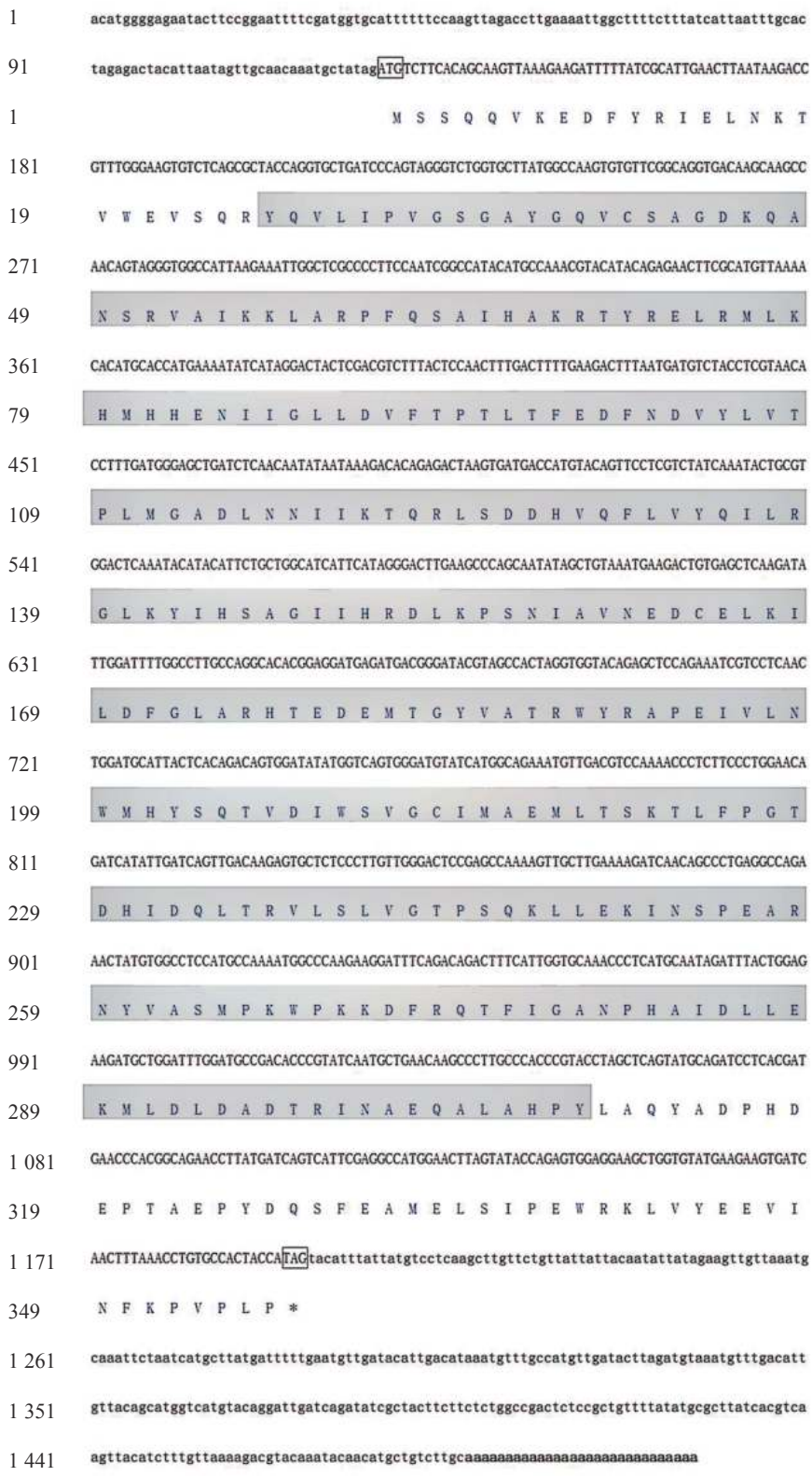


Fig. 1 Nucleotide sequence and deduced amino acid sequence of the pearl oyster *P. fucata. martensii MAPK p*38 cDNA

Mytilus galloprovincialis (Fig.4). The conserved spatial structure suggests that the potential link between protein structure and function.

2.2 Homology analysis of PmMAPK

The sequence similarity and sequence identity of

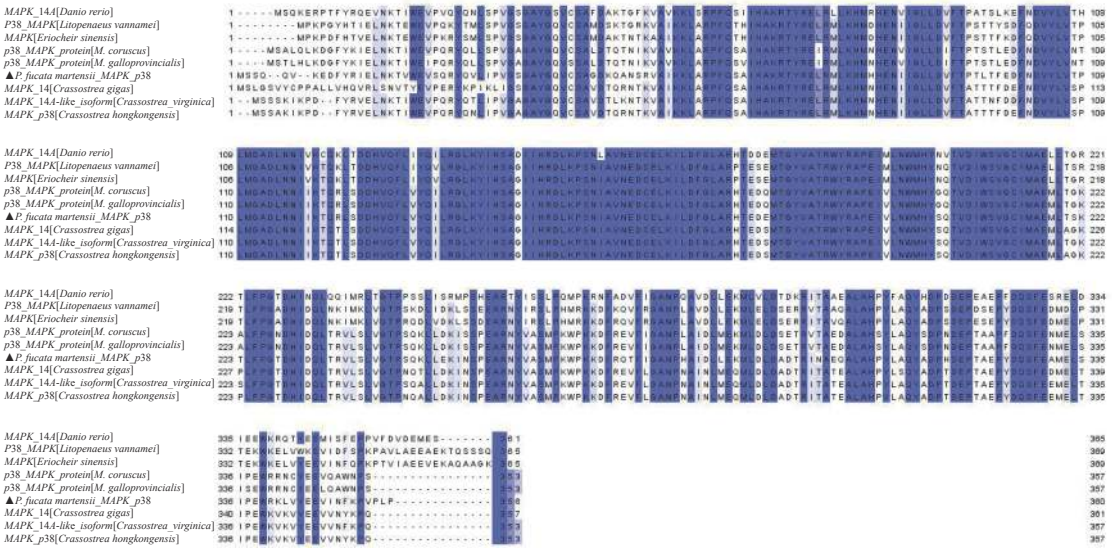


Fig. 2 Multiple sequence alignment analysis of *PmMAPK p*38 and other known sequences

Dark blue represents the same amino acids and light blue represents similar amino acids.

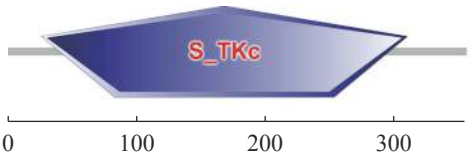
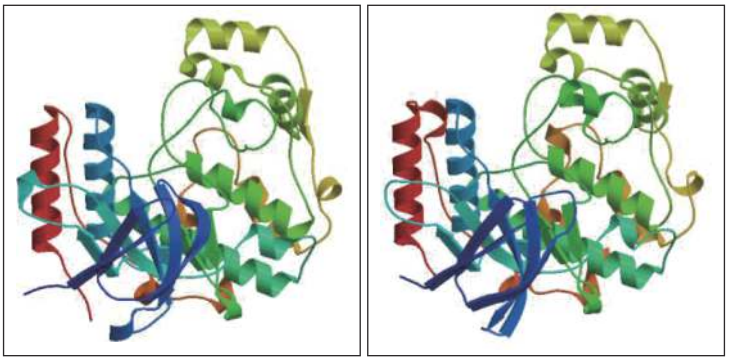


Fig. 3 The predicted serine/threonine kinase (S_TKc) domain (Pfam:CL0016) of pearl oyster *P . fucata martensii MAPK p*38

*PmMAPK p*38 with the *MAPK p*38 of different species ware determined and are presented in Fig. 5. The similarity and identity percentages ranging from 69.1%-90.4% and 59.6%-82.0%, respectively. The highest similarity and identity values belong to the *MAPK p*38 of *M. galloprovincialis* (90.4% and 82.0%, respectively), whereas the lowest similarity and identity values belong to the *MAPK p*38 of *Homo sapiens* (69.1% and 59.6%, respectively). Moreover, comparison of the *PmMAPK p*38 sequence with the MAPK sequence of *C. virginica*, *C. hongkongensis*, *M. coruscus*, *C. gigas*, *M. galloprovincialis*, *P. vannamei*, *E. sinensis* and *D. rerio* indicated a high percentage identity, suggesting that *PmMAPK p*38 is highly evolutionarily conserved.

Our phylogenetic analysis revealed a clear separation between the invertebrate and vertebrate MAPK p38 protein sequences, as illustrated in Fig. 6. Notably, PmMAPK p38 appeared to have a close relationship with the MAPK p38 of *M. galloprovincialis* and *M. yessoensis*. These relationships were consistent with conventional taxonomy.



(a) (b)

Fig. 4 The structure of PmMAPK p38 protein of *P. fucata martensii* (a) predicted by MAPK p38 protein of *M. galloprovincialis* (b) as a template (PDB: 3fkl)

percent identity

percent similarity	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1. *P. fucata martensii*		82.0	73.8	63.6	64.8	62.4	65.4	71.6	72.9	69.7	70.5	59.6	71.5	71.3
2. *Mytilus galloprovincialis*	90.4		70.0	62.1	64.0	58.0	63.2	68.9	69.6	68.5	68.8	59.2	68.5	68.8
3. *Mizuhopecten yessoensis*	84.5	82.8		59.2	59.9	57.8	61.9	67.6	67.6	66.0	66.8	57.2	67.3	67.3
4. *Apostichopus japonicus*	80.9	80.1	75.5		60.9	54.0	57.5	60.7	62.7	59.7	60.8	54.5	60.2	60.2
5. *Aurelia aurita*	78.7	78.9	74.9	77.8		56.5	58.7	63.6	66.9	64.4	66.3	56.9	64.6	64.6
6. *L. vannamei*	78.0	76.0	74.1	71.6	76.3		65.2	69.4	66.2	64.8	65.0	52.6	65.8	65.8
7. *Drosophila melanogaster*	79.8	79.0	77.4	72.7	74.0	78.7		74.7	66.4	63.7	65.3	55.0	65.8	65.8
8. *Bombyx mori*	85.0	84.2	81.2	78.9	78.3	81.9	86.1		71.2	68.7	70.4	58.5	70.9	70.9
9. *Xenopus laevis*	83.4	84.5	80.4	78.1	82.8	78.7	80.9	85.0		83.7	85.6	67.6	85.0	85.3
10. *Danio rerio*	83.9	83.7	81.2	77.8	79.8	78.4	77.6	82.0	90.9		87.0	64.9	88.9	89.2
11. *Salmo salar*	84.2	82.8	80.1	76.5	80.1	77.8	78.4	82.8	91.7	94.2		68.1	84.8	85.3
12. *Homo sapiens*	69.1	69.7	67.3	67.4	67.6	65.5	67.2	68.9	74.2	73.7	73.7		70.0	70.6
13. *Rattus norvegicus*	83.3	84.2	80.4	78.6	80.8	79.2	78.4	82.2	92.8	94.5	90.9	76.1		99.2
14. *Bos taurus*	83.3	84.4	80.7	78.6	80.6	79.4	78.7	82.5	93.1	94.7	91.1	76.4	99.4	

Fig. 5 Similarity and identity of MAPK p38 whole sequences among species, including vertebrates and invertebrates

The percents of identity are shown in upper triangle and the percents of similarity are shown in the lower triangle; the red indicates the highest similarity; *Mytilus galloprovincialis* (AGW27418.1), *Mizuhopecten yessoensis* (ANG60948.1), *Apostichopus japonicus* (ASU91381.1), *Aurelia aurita* (ARJ54259.1), *L. vannamei* (AGG82488.1), *Drosophila melanogaster* (O62618.1), *Bombyx mori* (NP001036996.1), *Xenopus laevis* (NP001080300.1), *Danio rerio* (ABL68016.1), *Salmo salar* (ABL68016.1), *Homo sapiens* (Q16539.3), *Rattus norvegicus* (NP112282.2), *Bos taurus*(NP001095644.1).

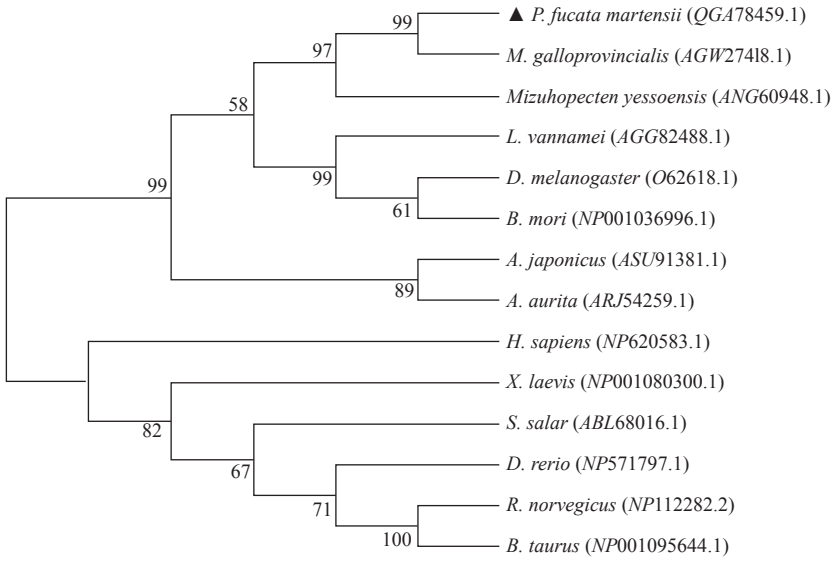


Fig. 6 Neighbor-joining (NJ) phylogenetic tree constructed based on MAPK p38 amino acid sequences

The triangle symbol (▲) refers to the MAPK p38 of *P. fucata martensii*.

2.3 Expression level of *PmMAPK p38* in different tissues and inresponse to stress

The expression of *PmMAPK p38* mRNA was examined using qPCR in five different types of healthy *P. fucata martensii* tissue. The results illustrated that the hepatopancreas exhibited the highest level of *PmMAPK p38* expression, followed by the hemocytes, mantle, and gills. Whereas the adductor muscles displayed the lowest *PmMAPK p38* expression (Fig.7).

To investigate further the immune response function of *PmMAPK p38* , we conducted qRT-PCR

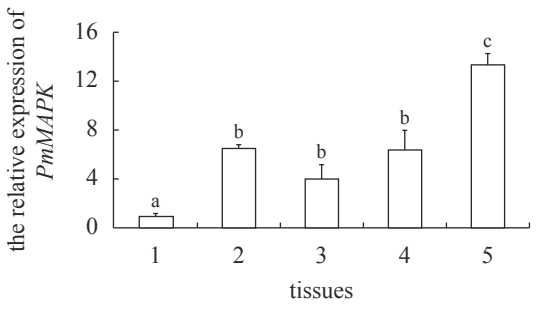


Fig. 7 Expression of *PmMAPK p38* mRNA in five representative sample tissues of *P. fucata martensii*

1. adductor musle, 2. mantle, 3. hemocytes, 4. gill, 5. hepatopancreas; error bars represent the mean±SE (n=3); the significant difference was indicated by different letters over the bars (P<0.05).

analysis to determine the temporal expression of the *PmMAPK p*38 mRNA under *V. harveyi* and LPS challenge in haemocytes. The results showed that *PmMAPK p*38 in *P. fucata martensii* was subject to differences in response to different stimuli (Fig.8). Under LPS stimulation, the relative expression of *PmMAPK p*38, after 2 h of stimulation, was higher than that stimulated by *V. harveyi,* but 4-24 h after stimulation, the *PmMAPK p*38 expression level through *V. harveyi* infection was higher than that with LPS stimulation. In the *V. harveyi* infection group, *PmMAPK p*38 expression was significantly increased at 2 h and 4 h and peaked at 2 h ($P<0.05$). *PmMAPK p*38 expression subsequently, reaching its lowest at 8 h, and then increased gradually, reaching the control level at 24 h (Fig.9). In the LPS stimulation group, *PmMAPK p*38 expression was upregulated and reached the maximum level at 2 h ($P<0.05$), followed by a decrease to the level of the control at 4 h, sustaining that same level until the end of the experiment (24 h) (Fig.10).

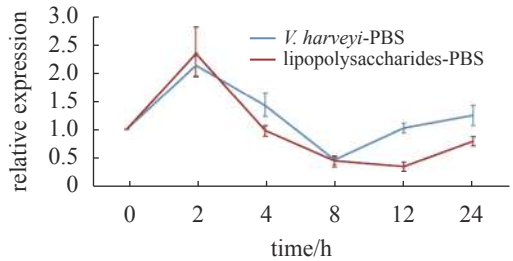


Fig. 8 Expression of *PmMAPK p*38 in hemolymph under two different stimuli of *V. harveyi* and LPS

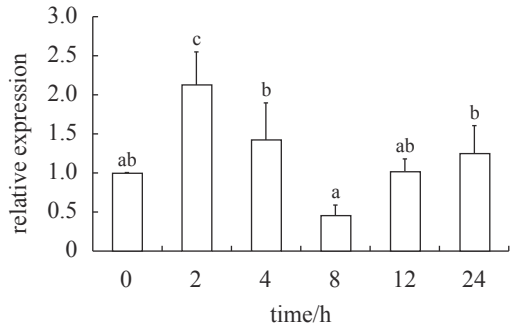


Fig. 9 Relative expression of *PmMAPK p*38 in hemocytes after *V. harveyi* stimulation

V. harveyi was used as the experimental group and PBS as the control group; the mRNA expression levels of *PmMAPK p*38 in the 2 groups at different time points were detected, and the relative expression of the two groups was calculated to obtain the results; the data with the same superscript means no significant difference between them ($P>0.05$).

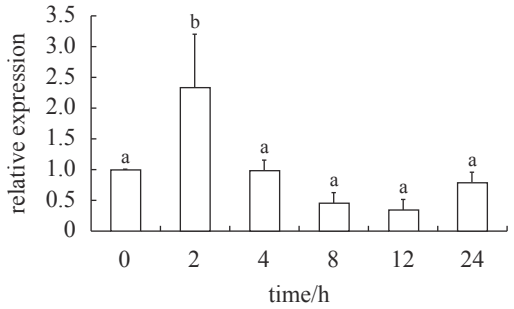


Fig. 10 Relative expression of *PmMAPK p*38 in hemocytes after LPS stimulation

LPS was used as the experimental group and PBS as the control group; the mRNA expression levels of *PmMAPK p*38 in the 2 groups at different time points were detected, and the ratio of the 2 groups was calculated to obtain the results; the data with the same superscript means no significant difference between them ($P>0.05$).

3 DISCUSSION

P38 mitogen-activated protein kinases are mitogen-activated protein kinases (MAPKs) that respond to stress stimuli, such as cytokines, ultraviolet irradiation, heat shock, and osmotic shock, and involved in cell differentiation, apoptosis, and autophagy. The p38 MAPK family members share a serine/threonine-protein kinase (S_TKc) domain and a conserved Thr-Gly-Tyr (TGY) motif in their activation loop. The protein kinase function is conserved from *E. coli* to humans [23], and phosphorylation leads to functional changes in the target protein. The TGY motif is essential for the activation of p38 MAPK [24]. Our study demonstrated that *PmMAPK p*38 shares high homology with the *MAPK* p38 gene of other species and has a conserved S_TKc domain and TGY motif, consistent with earlier findings [25], revealing for the first time the presence of *PmMAPK p*38 in *P. fucata martensii*.

In invertebrate innate immunity, both humoral and cellular immunity play crucial roles as the first line of defense against foreign invasion [4]. Hemolymph, which comprise miRNAs[26], enzymes, and proteins[27], is particularly important due to *P. fucata martensii*'s open vascular system that makes it vulnerable to bacteria and viruses [28]. Thus, investigating the role of hemolymph in *P. fucata martensii*'s immune response is necessary. As MAPK is well-characterized in hemolymph, we analyzed the expression patterns of *MAPK p*38 for further under-

standing of its physiological functions. We found that *MAPK p*38 was expressed in all sampled tissues, with the highest expression detected in hepatopancreas, followed by mantle edge, gill, hemolymph and adductor muscle respectively (Fig.7), indicating a strong tissue-specific expression pattern. In other species like salmon, the *MAPK p*38 mRNA is widely distributed in organs such as the head, kidney, and spleen. In contrast, in the ovary, *MAPK p*38 mRNA is highly expressed, indicating a specific role [29]. In blunt snout bream, *MAPK p*38 mRNA is expressed in all analyzed tissues, including the brain, heart, muscle, intestine, blood, kidney, liver, gill, head kidney and spleen, but the spleen shows the highest level of expression[30]. In humans, the *MAPK p*38α mRNA is highly expressed in various organs such as the placenta, cerebellum, bone marrow, thyroid, peripheral blood leukocytes, liver and spleen, while *p*38*δ* is highly expressed in the salivary gland, pituitary gland, and adrenal gland[31]. The diverse expression profiles indicate different regulatory functions of MAPK in distinct species. The expression of *MAPK* in *P. fucata martensii* immune organs suggests its critical role in resisting disease invasion.

It has been reported that pathogen infections can activate the p38 MAPK signaling pathway [32]. Studies demonstrate the significant of *MAPK p*38 in molluscan innate immunity. For example, in *C. hongkongensis, MAPK p*38 mRNA expression was enhanced significantly by pathogen challenge, with the highest level in the *V. alginolyticus* group (7.4-fold) and *S. haemolyticus* (12.2 -fold) at 24 h post-challenge compared to the control [33]. However, in the Yesso scallop *P. yessoensis*, *MAPK p*38 elicited no significant immune response to *Micrococcus luteus* (Gram-positive bacteria) or *V. anguillarum* (Gram-negative bacteria) [34]. In *M. galloprovincialis*, *V. parahaemolyticus* Conero induced transient phosphorylation of p38 MAPK, with a peak at 15-30 min. In contrast, the response to *V. vulnificus* 509 was slow but persistent with extremely high phosphorylation of p38 MAPK [35]. In clams, the level of *MAPK p*38 phosphorylation correlated positively with PO expression upon *Vibrio* stimulation[36]. Our infection experiment showed that *PmMAPK p*38 expression in the LPS and HJ-infected hemolymph peaked at 2 h, significantly different from previous reports [33-35]. This could result slight differences in *p*38 expression in different populations and the rapid expression of *PmMAPK p*38 in the hemolymph upon pathogen invasion. The sensitivity of different bivalves to hemolymph killing may be due to varying metabolites or characteristic molecules of bacteria, specific conditioning molecules, blood cell-binding and phagocytosis capacity towards different bacteria, and bacterial sensitivity to intracellular killing [37].

In all, our study shows that *MAPK p*38 can generate an immune response to pathogen stimulation. The results will be the foundation for detailed investigations regarding *P. fucata martensii* and the basic strategy via which bacteria cause infection and disease in these bivalves. However, the present results are insufficient to understand the regulatory mechanism of *MAPK p*38. In the future, we will explore the mechanism via which *MAPK p*38 regulates downstream targets, including several kinases, transcription factors, and cytosolic proteins, as well as how the different components of the *MAPK p*38 pathway interact with each other.

（作者声明本文无实际或潜在的利益冲突）

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马氏珠母贝丝裂原活化蛋白激酶 p38 的克隆与表达

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摘要: 丝裂原活化蛋白激酶（mitogen-activated protein kinase, MAPK）是一种丝氨酸/苏氨酸蛋白激酶, 该蛋白激酶通过磷酸化级联介导的信号通路在细胞对细胞外刺激的反应中起着重要作用。本研究利用 cDNA 末端快速扩增 (rapid amplification of cDNA ends, RACE) 技术克隆获得马氏珠母贝 *MAPK p*38 (*PmMAPK p*38) cDNA 全长序列并对其序列进行生物信息学分析; 利用实时荧光定量 PCR (qPCR) 技术分析了 *PmMAPK p*38 在马氏珠母贝不同组织以及不同免疫刺激后的表达水平。结果显示, *PmMAPK p*38 cDNA 全长为 1 516 bp, 开放阅读框长度为 1 071 bp, 共编码 356 个氨基酸, 理论分子量为 40.88 ku; 结构域预测结果表明 *PmMAPK p*38 含有 MAPK 家族典型的 S_TKc 结构域; 多序列比对、进化树构建以及 MatGAT 计算结果显示 *PmMAPK p*38 与其他物种的相似度、保守程度较高; 荧光定量分析结果表明, 该基因在马氏珠母贝中存在广泛表达, 在肝胰腺中表达量最高, 其次为外套膜, 最低是闭壳肌。马氏珠母贝在受到 LPS 刺激后, *PmMAPK p*38 的相对表达量在刺激后 2 h 达到最高, 12 h 降到最低, 最高约为最低的 5 倍; 而在哈维氏弧菌刺激后, *PmMAPK p*38 的相对表达量在 2 h 达到最高, 8 h 降到最低, 最高约为最低的 4 倍。研究表明, *PmMAPK p*38 可能在马氏珠母贝的免疫反应, 尤其是在抵御外部细菌侵入中起着重要的作用。本研究为贝类免疫防御系统的研究提供了基础资料。

关键词: 马氏珠母贝; *MAPK p*38; 免疫; 基因克隆

中图分类号: Q 785; S 944.3 **文献标志码:** A