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Separation, identification and gene expression analysis of PmAMP-1 from *Pinctada fucata martensii*

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ABSTRACT

Antibacterial peptides (AMPs) constitute an important part of the body's innate immune system and are responsible for a wide range of inhibitory effects against pathogens such as bacteria, fungi, and viruses. In this study, multi-step high performance liquid chromatography (HPLC), combined with Mass Spectrometry (MS), was used to isolate and identify proteins with antibacterial activity from the serum of *Pinctada fucata martensii* (*P.f. Martensii*) and obtain a component named *P.f. Martensii* antimicrobial peptide-1 (PmAMP-1). *PmAMP-1* cDNA was cloned and sequenced by rapid amplification of cDNA ends (RACE) and mRNA expression of was analyzed by quantitative real-time PCR (qRT-PCR). From the results of this study, full-length *PmAMP-1* cDNA was shown to be 700 base pairs (bp) long with an open reading frame (ORF) of 294 bp, encoding 97 amino acids with a predicted structure that is mostly α-helices. *PmAMP*-1 mRNA was constitutively expressed in all tested tissues including the adductor muscle, mantle, hepatopancreas, gill, gonads and hemocytes. The highest level of *PmAMP*-1 transcription was observed at 8 h and 2 h after bacterial challenge in hemocytes and adductor muscle ($p < 0.01$), respectively. Furthermore, PmAMP-1 caused significant morphological alterations in *E. coli,* as shown by transmission electron microscopy (TEM). The results from this study provide a valuable base for further exploration of molluscan innate immunity and immune response.

1. Introduction

Antibacterial peptides (AMPs) are a class of small peptides which exhibit rapid action, have both broad antibacterial and strong bactericidal activity, high stability, and carry a low risk of targets developing resistance to them [1,2]. They can attack bacteria and fungi, as well as protect against viruses and parasites [3–5]. AMPs exist in all living creatures and represent the first line of host defense against infectious pathogens and AMPs from many different organisms have been described in studies over the years [6,7]. Tachyplesin was first isolated and purified in 1998 from marine organisms by Nakamura [8] and other AMPs have been successfully isolated and purified from marine species such as *Litopenaeus vannamei*, *Epinephelus fasciatus, Misgurnus anguillicaudatus* and bivalves [9–12]. AMPs have been shown to be one of the main immune factors in fish, shrimp, and shellfish. Current research on the AMPs of marine organisms has focused mostly on lower organisms such as mollusks and crustaceans. AMPs are the major component of the innate immune system in mollusks [13–15] and their antibacterial activity was first reported in the '80s [16]. The study of AMP's in mussels have found that in *Mytilus edulis*, *Mytilus galloprovincialis and Mytilus coruscus*, eight families of antimicrobial peptides, including Defensins [17], Myticins [18], Mytilins [19], Mytimycins [20], Big defencin [21], Macin [22], Myticusin [23] and Mytichitin [24] have been identified. Several new AMPs have recently been isolated from other marine mollusks [25,26].

AMPs can be broadly divided into three types according to their molecular architecture; the first contains alpha helixes, the second contains disulfide bonds and β-sheets, while the third consists of random coils rich in hydrophobic amino acids [6,27,28]. The eight AMP family members identified in the genus *Mytilus* belong to the second type.

While it is generally accepted that the mechanism of action of AMPs involves interactions between hydrophobic amino acids of the AMP and the membrane of target cells which leads to disrupting membrane integrity, there are different theories on how AMPs enter the cell membrane. In Christensen's model, several AMP amino acids are first adsorbed on to the surface of the membrane which then allows other hydrophobic amino acids to directly enter the membrane and form ion channels which lead to cell membrane degradation [29]. With Fink's model, when AMPs interact with the cell membrane, first the N-terminus comes into contact with the membrane and then only the hydrophobic portion of the C-terminus is able to directly enter it [30].

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Lastly, in Clague's model, AMPs interact with membrane proteins. This leads to protein aggregation and inactivation, and eventually channels forming in the cell membrane [31]. After AMPs have disrupted and entered through the membrane, there may be further binding targets within the cells. There are a number of intracellular bactericidal mechanisms including:disruption of nucleic acid interactions, inhibition of synthesis of nucleic acids and/or proteins, enzyme inhibition, and downregulation of genes essential for membrane formation [32]. As opposed to traditional antibiotics, pathogens rarely develop resistance to the AMPs produced by marine organisms, and as such researchers have turned to them as the most likely candidates to replace antibiotics.

In the marine environment, where microbial growth is relatively dense, AMPs are the most effective defense against pathogens for fish, shrimp, and shellfish [33], and while this has become a rapidly expanding research area, the precise mechanisms of action and phylogenetic status of AMPs are still unclear.

Pinctada fucata martensii (*P.f. Martensii*) is a bivalve mollusk, and as it lacks a specific immune system its defense against pathogens relies entirely on cellular immunity and humoral immune factors. Due to the deterioration of coastal water quality, various diseases have occurred more frequently in recent years, resulting in increased mortality of *P.f. Martensii* and causing serious economic losses for China's pearl industry [34,35]. Improving the disease resistance of *P.f. Martensii* is therefore an urgent concern that needs to be addressed.

2. Materials and methods

2.1. Animals and hemolymph collection

Pearl oysters (*P.f. Martensii*), aged about 2 years old with shell lengths ranging from 5 to 6 cm, were collected directly from the sea in Chengwu, Zhanjiang, Guangdong Province, China. Animals were cultured at 25–27 °C in tanks with recirculating seawater for three days prior to experimentation. Oysters were challenged with a mixture of heat-killed *Escherichia coli* and *Micrococcus luteus* injected into the adductor muscle with a needle, and then returned to sea water. Hemolymph was collected and pretreated using the methods described by Mitta [36]. The plasma was clarified by centrifugation (3000 rpm, 20 min, 4 °C) and the supernatant kept at −80 °C prior to use.

2.2. Peptide purification

Purification was performed on a Waters Delta 600 HPLC system equipped with a Waters 2487 absorbance detector and two types of reversed-phase columns: a Sunfire™ prep C18 column (10 × 250 mm Waters) and an analytical Vydac C18 RP HPLC column (218TP54, 4.6 × 250 mm).

First, plasma samples were fully eluted with 5%, 18%, 40%, and 60% acetonitrile containing 0.05% TFA (solution B) using a Sunfire™ prep C18 column at a flow rate of 1 mL/min. The eluted fractions were manually collected by varying the concentration of acetonitrile, after which the components were freeze-dried and subjected to an antibacterial activity test. Next, active fractions were loaded onto an analytical Vydac C18 RP HPLC column, eluted with a gradient of 5–20% acetonitrile containing 0.05% TFA for 5 min, 20–60% acetonitrile for 45 min, and 60–95% acetonitrile for a further 5 min. The fractions with peaks were then manually collected and tested for antibacterial activity by freeze drying. The strains used in the test were *E. coli* and *M. luteus*.

2.3. Detection of antibacterial activity

The antibacterial activity of each eluted fraction was determined using the method of Charlet [19]. *E. coli* and *M. luteus* were cultured in LB liquid medium to logarithmic growth phase, and then diluted with the LB to an optical density value OD600 of 0.001. Subsequently, 90 μL of bacterial solution and 10 μL of sample were added to each well of a previously sterilized 96-well plate and mixed well (the sample powder was diluted with ultrapure water). Deionized water was used as a negative control. The mixture was then incubated at 37 °C for 12 h and the OD600 value of the sample before and after sample addition was measured using a microplate reader. Three sets of parallel tests were carried out for each sample.

2.4. Mass spectrometric identification of active peptides

The molecular weight of the active protein was identified using SDS-PAGE and the amino acid sequence of the trypsin-digested fragment was analyzed by secondary mass spectrometry (AB SCIEX, USA) combined with de novo sequencing. Homologous sequences were identified in the NCBI protein database and the *P.f. Martensii* genome using the partial sequence of the purified PmAMP-1 as a query. Primers were designed using the highest-ranking homologous sequence as a template to perform cDNA cloning.

2.5. Sequence analysis and structural modeling

The cDNA sequence of PmAMP-1 was analyzed for similarity with other known sequences using BLAST (http://www.ncbi.nlm.nih.gov/BLAST/) and the amino acid sequence was analyzed using the following resources accessed via the ExPASy portal (http://www.expasy.org/). The open reading frame (ORF) was characterized using the ORF Finder (http://www.ncbi.nlm.nih.gov/gorf/orfig.cgi) and the theoretical isoelectric point and molecular mass were estimated with ExPASy (https://web.expasy.org/protparam/). The secondary structure was predicted with SOPMA (https://npsa-prabi.ibcp.fr/cgi-bin/npsa_automat.pl?page=/NPSA/npsa_sopma.html) and the tertiary structure of PmAMP-1 was built by homology modeling using the Swissmodel server (https://swissmodel.expasy.org/).

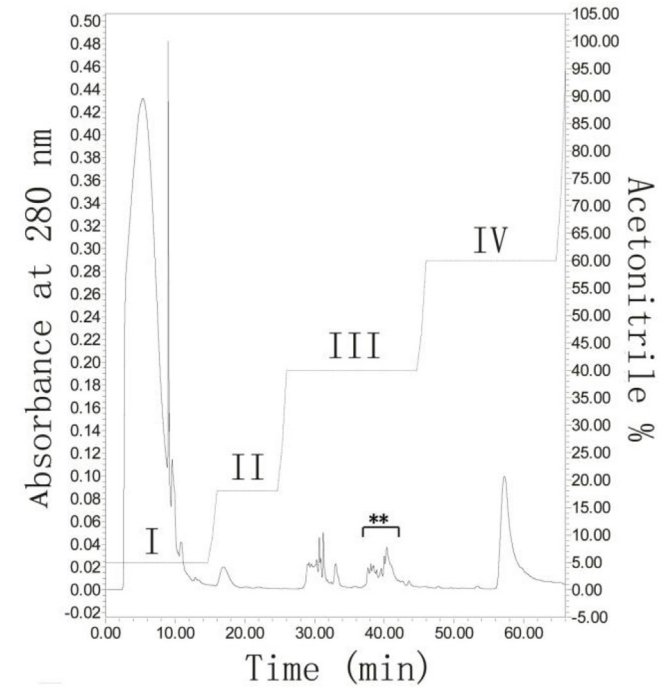
2.6. Quantitative analysis of PmAMP-1 expression

The tissue specific expression of *PmAMP-1* transcripts and the temporal expression of *PmAMP-1* transcripts in hemocytes and adductor muscle of *P.f. Martensii* challenged with inactivated *E. coli* and *M. luteus* was determined by qRT-PCR. The tissues obtained included adductor muscle, mantle, hepatopancreas, gill, gonads and hemocytes. The total RNA of each tissue was extracted and reverse transcribed into cDNA. The expression of *PmAMP-1* in tissues was measured using an Applied Biosystem 7500 Real-time PCR System. Two *PmAMP-1* specific primers, PmAMP-1-RT-F (5′-CACAGCAAAGGCAGATGGATAC-3′) and PmAMP-1-RT-R (5′-CTTTGTCGTCCAGGGTGTCG-3′) were used to amplify the gene fragment, which was sequenced to verify its identity.

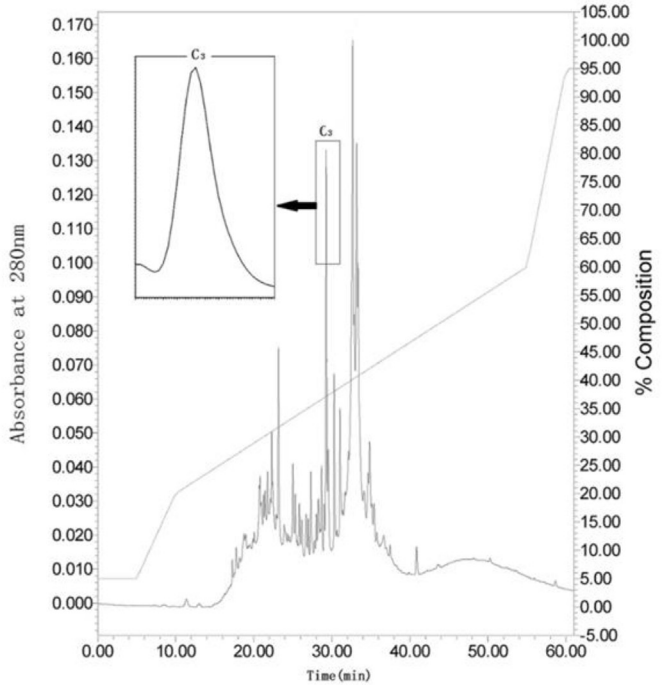
For the bacterial challenge experiment, 120 pearl oysters were randomly divided into two groups. The experimental group was injected intramuscularly with 30 μL of a mixture of inactivated *E. coli* and *M. luteus*, whereas the control group was injected with 30 μL phosphate buffered saline. A total of nine post injection time points, 0, 2, 4, 8, 12, 16, 24, 36 and 48 h, were selected for expression analysis.

2.7. Transmission electron microscopy (TEM)

The sample of PmAMP-1 was dissolved in deionized water and 200 μL of exponential-phase *E. coli* was treated with 20 μL of the peptide for 60 min at 37 °C. PBS replaced PmAMP-1 as the control. The cells were pelleted by centrifugation at 3000 rpm for 10 min at room temperature and the bacterial pellets were then fixed in 200 μL 3% glutaraldehyde overnight. After treatment, 200 μL 2% sodium phosphotungstate aqueous solution was added to the bacterial suspension which was dropped on copper grids. The sample was air dried after using filter paper to remove the residual water for 5 min. Microscopy was performed with a JEM-1230 JEM-1400 (Japan Electronics Corp) microscope under standard operating conditions.



(A)



(B)

Fig. 1. Purification and identification of PmAMP-1. (A) The plasmasample collected from *P.f.martensii.*was separated using a SunfireTM prepC18columnand the target fraction (marked by"**") with antibacterial activity was subjected to further purification. (B) Further purification of the target fraction by analytical Vydac C18 column and the eluate with antibacterial activity (denoted by C3) was collected for structural analysis.

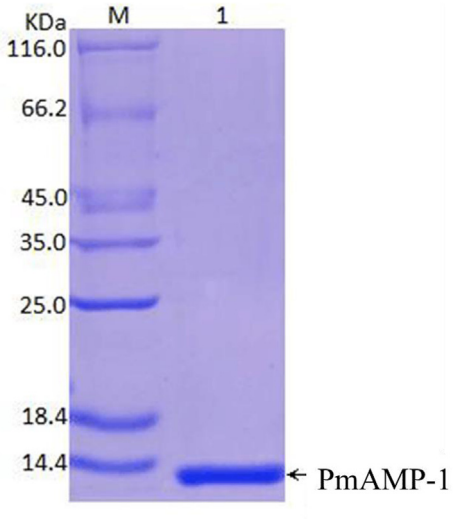


Fig. 2. SDS-PAGE of PmAMP-1.Lane M: protein molecular marker; Lane 1: the protein with antibacterial activity.

3. Results

3.1. Isolation and identification of PmAMP-1

Hemolymph was collected from *P.f. Martensii*. Cells and debris were removed by centrifugation and the supernatant was loaded on to a Sunfire™ prep C18 column and eluted with a gradient of 5–60% acetonitrile, yielding the chromatogram shown in Fig. 1A. Four fractions are shown and the "**" represents a fraction having antibacterial activity.

The fractions denoted by a "**" mark were subjected to analytical reversed phase HPLC using a Vydac C_{18} RP HPLC column. Antimicrobial activity was monitored from aliquots of each fraction. One of the purified proteins that eluted at approximately 29 min (Fig. 1B, labelled C3) had bacteriostatic activity. The molecular mass of this protein is approximately 11 kDa (Fig. 2). The protein was sequenced and the following partial amino acid sequences were obtained: ADGYKEYTINR and VIGIDLDGDIVER, as shown in Fig. 3 (A and B).

3.2. Antibacterial activity of PmAMP-1

The antibacterial activity of PmAMP-1 was determined using the microplate reader method. After the addition of samples (The concentration of PmAMP-1 was 200 μg/mL) to *M. luteus* and *E. coli*, the OD values at 0 h and 12 h were measured. As can be seen from Fig. 3, the growth of *M. luteus* (Fig. 4A) and *E. coli* (Fig. 4B) was significantly inhibited following the addition of PmAMP-1 ($p < 0.05$).

3.3. Characterization of PmAMP-1

The cDNA sequence named *PmAMP-1* was determined. The full-length fragment was 700 bp including a 156 bp 5′ untranslated region (UTR) and a 250 bp 3′ UTR. The sequence was deposited to GenBank with accession no.MN053909. The *PmAMP-1* cDNA contains an open reading frame (ORF) of 294 nucleotides encoding 97 amino acids and the predicted molecular mass of the translated polypeptide chain is 11.03 kDa with a theoretical isoelectric point (pI) of 4.58. The most prevalent amino acids in PmAMP-1 are aspartic acid, threonine, lysine, isoleucine and leucine. The amino acid sequences identified by mass spectrometry were found in the protein sequence encoded of PmAMP-1, as shown by the black underlines in Fig. 5. The online prediction of the PmAMP-1 domains using SMART revealed that no functional domains are present and that it is considered to be a new protein with

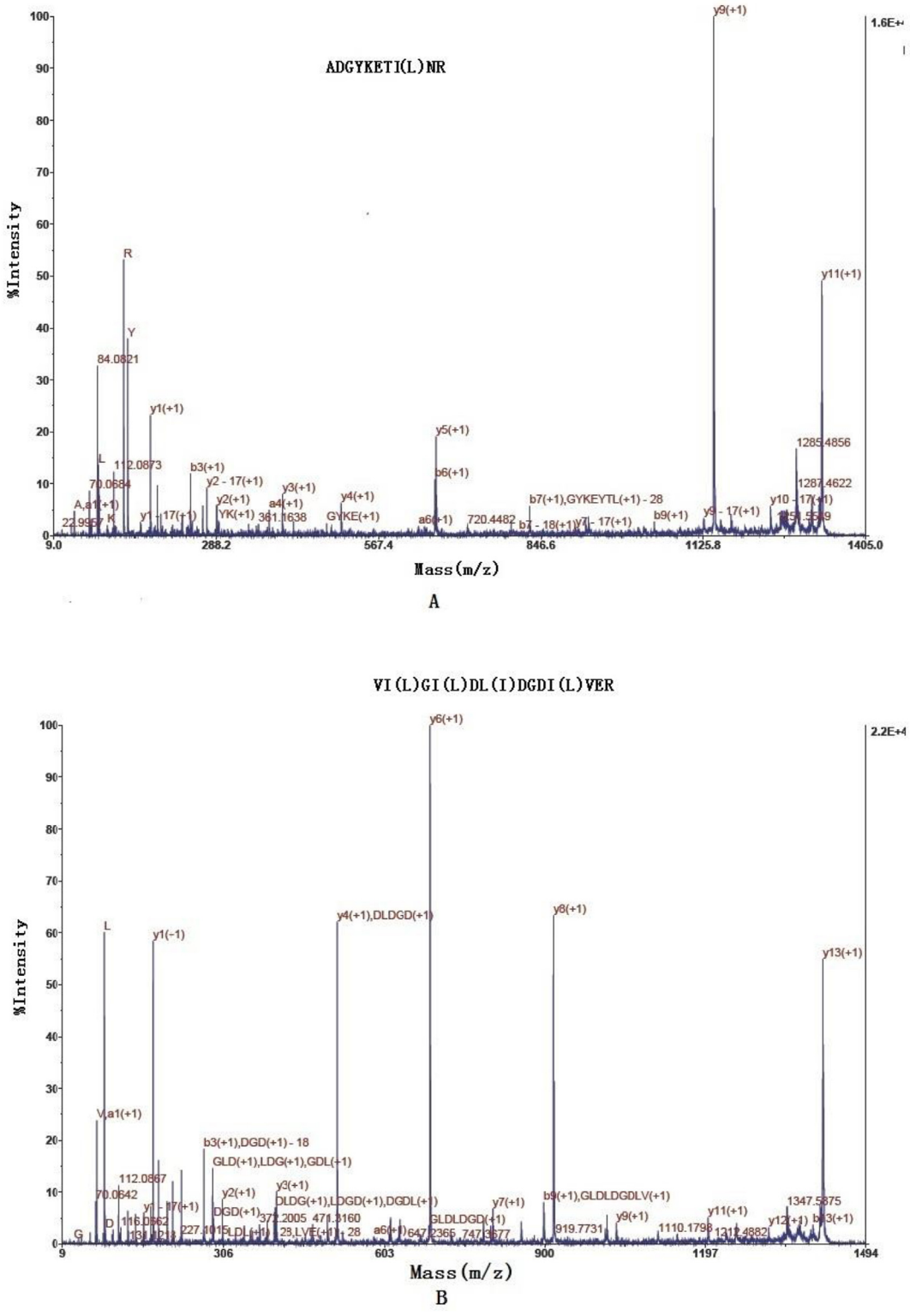


Fig. 3. Mass spectrum of the PmAMP-1 peptide fragment.

antibacterial activity. The predicted secondary structure suggested that PmAMP-1 contains a mix of random coils, extended strands and α-helices.

The tertiary structure of PmAMP-1 was built by homology modeling using the SWISSMODEL server. 17 templates were found and 2 models have been built. We chose the model with the highest coverage, 0.67 (from the 24–91 amino acids) with 20% similarity and GMQE of 0.39, as a template. The structure of the Ubiquitinating/deubiquitinating enzyme SdeA (Protein Data Bank [PDB] accession no. 5ysk) was used as a template. The predicted tertiary structure of PmAMP-1 contains four alpha helices (Glu25-Lys37, Thr39-Leu44, Glu48-Ile56, and Lys67-Phe74) and two random coils (Asp58-Asp64, Thr81-Ile86).

3.4. Tissue expression analysis of PmAMP-1

Expression levels of *PmAMP*-1 mRNA in the adductor muscle,

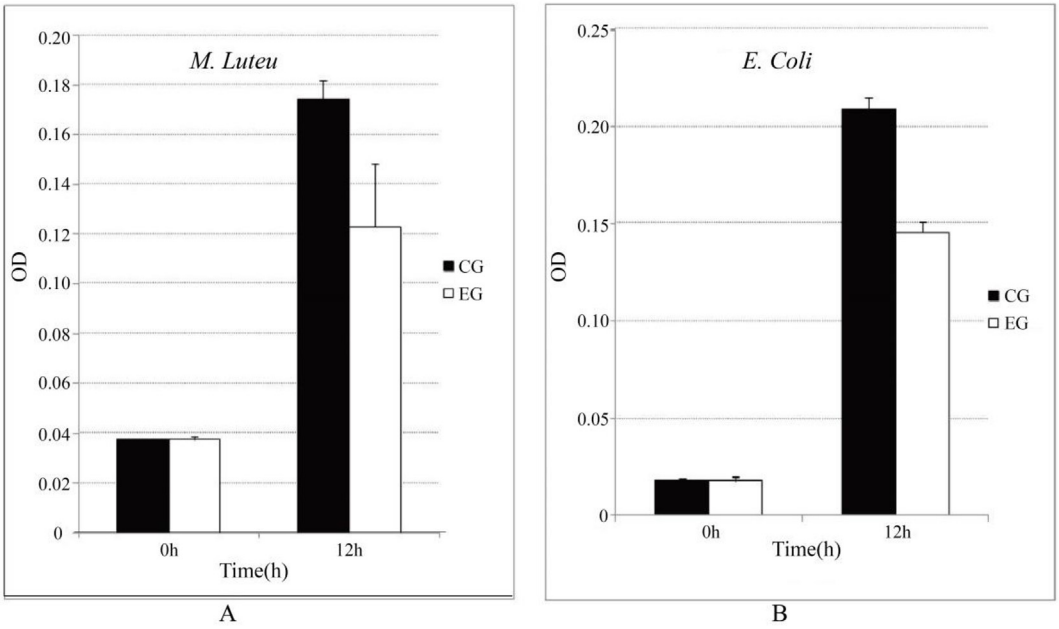


Fig. 4. Inhibition assay of PmAMP-1 for *M. luteus*and *E. coli*.A for*M. luteus*and Bfor*E.coli.*, CG represent control group and EG represent experimental group.

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1   ACATGGGGGACCTAGTCAGCATCTGTGATACAGGCAGTAATAAGGTGAGGGGATACGTCC
61  CTTACAGGAGTATAAACACAGACGTGACACGGATACGGCGTGAGGACTGCATCACTGACC
121 AAGATCTATATAGAAACTGACGTAGAATAGTTCACCATGGATACGATGATTGACAGTCTC
1                                       M  D  T  M  I  D  S  L
181 AACAAGGATATGTGGGCTGAAACCACAGCAAAGGCAGATGGATACAAAGAATATACCATC
9   N  K  D  M  W  A  E  T  T  A  K  A  D  G  Y  K  E  Y  T  I
241 AACAGGCAGCACAAACGTATAGATAAAACCACACTAGGGCTTTTCCTGGATCCAGAGGAG
29  N  R  Q  H  K  R  I  D  K  T  T  L  G  L  F  L  D  P  E  E
301 GGCGACTCAGTCACAGTGCAAATTAACGACACCCTGGACGACAAAGATCCACTCAAAAAT
49  G  D  S  V  T  V  Q  I  N  D  T  L  D  D  K  D  P  L  K  N
361 ATCTGTAGAGCAGAATTCAAACTTGACCCCACCAATACCAAGGTAATAGGTATCGACCTT
69  I  C  R  A  E  F  K  L  D  P  T  N  T  K  V  I  G  I  D  L
421 GACGGTGACATCGTCGAACGGAAGAGTTAGAGAGTTAGGACACAGGAAGTCACGTGACCA
89  D  G  D  I  V  E  R  K  S  *
481 ATACCGAGTGTTCAGTGGAACCTTTTCACCCGGAAATGAAAGCCCTATGTTGACTAGTCC
541 AGATCAGCTGTACAAATCTCTTGGAATAATTTATCCATACCATATGTTCCCATACGTTCA
601 TTATGAAACAATGGTGTGTTTTGTATGTATTTGTGTGCAAAGCATAGTATATGTCAGTAA
661 AATCTTTACATGCAAAAAAAAAAAAAAAAAAAAAAAAAA
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Fig. 5. cDNA and deduced amino acid sequence of PmAMP-1. The open reading frame (ORF) is indicated in capital letters and 5′ UTR or 3′ UTR are indicated by lowercase letters, the double underline is representative of a poly A polyadenylation signal, and the underlined amino acids indicate the obtained amino acid sequencefrom MS.

mantle, hepatopancreas, gill, gonads and hemocytes of *P.f. Martensii* were analyzed by qRT-PCR. As shown in Fig. 6A, *PmAMP*-1 mRNA was constitutively expressed in all examined tissues, with the highest expression level found in the adductor muscle and weak expression in hemocytes.

After bacterial challenge, the expression level of *PmAMP*-1 mRNA in hemocytes was upregulated and reached a maximum level at 8 h with a 14-fold increase compared to the control ($p < 0.01$). After 36 h, the lowest levels were obtained with only a 0.07-fold increase compared with the control ($p < 0.01$) (Fig. 6B).

As shown in Fig. 6C, the expression level of *PmAMP*-1 mRNA in the adductor muscle was upregulated, and reached a maximum level at 2 h post-infection (4.96-fold, $P < 0.01$).

3.5. Alterations in the surface morphology of E. coli

To further investigate the mechanism of action of PmAMP-1, TEM was employed to observe the morphology of *E. coli* cells in the presence or absence of PmAMP-1. The final concentration of the purified PmAMP-1 used in this experiment was 1 mmol/L. As shown in Fig. 7, the exterior of the control *E. coli* cells (Fig. 7-A) was clear and intact, with three dense structures present inside. After exposure to PmAMP-1 (Fig. 7-B), partial dissolution of both ends of *E. coli* cells was observed (shown by the arrow in Fig. 7-B), and the internal structures were also significantly altered compared with the control group. Simultaneously, a vesicular structure appeared on the surface of the cell wall (Fig. 7-C, D), indicating that the cell wall had disintegrated to cause a local release of the cell contents.

4. Discussion

AMPs are one of the main immunological means for shellfish to resist the invasion of pathogenic microorganisms. The shellfish antimicrobial peptides currently studied have been mainly separated and purified from plasma and gill. At present, study of the AMPs of *P.f. Martensii* is limited. In this experiment, PmAMP-1 was isolated and characterized from the hemolymph of *P.f. Martensii*. The molecular weight of PmAMP-1 was predicted to be 11 kDa. The molecular weight of antimicrobial peptides identified from shellfish is mainly between 3000 and 5000 Da, with a few proteins that are over 10000 Da. For example, Mytilin [19], Myticin [18], and Defensin [17], which have been isolated and identified from *Mytilus* species, have a molecular weight of approximately 4000 Da and their sequences usually contain 3-4 pairs of disulfide bonds to form a dense α/β domain. The molecular weight of Myticusin from *Mytilus coruscus* is over 10,000 Da [19]. PmAMP-1 is remarkable for the large number of charged residues (35), with 14 positively charged residues and 21 negatively charged residues. Because there are more negatively charged residues than positively charged residues, especially 15 aspartic acid residues, the isoelectric point was 4.58. This is somewhat surprising as the other known mussel AMPs (defensins, mytilins, myticins and mytimycins) are classical cationic peptides [16,37].

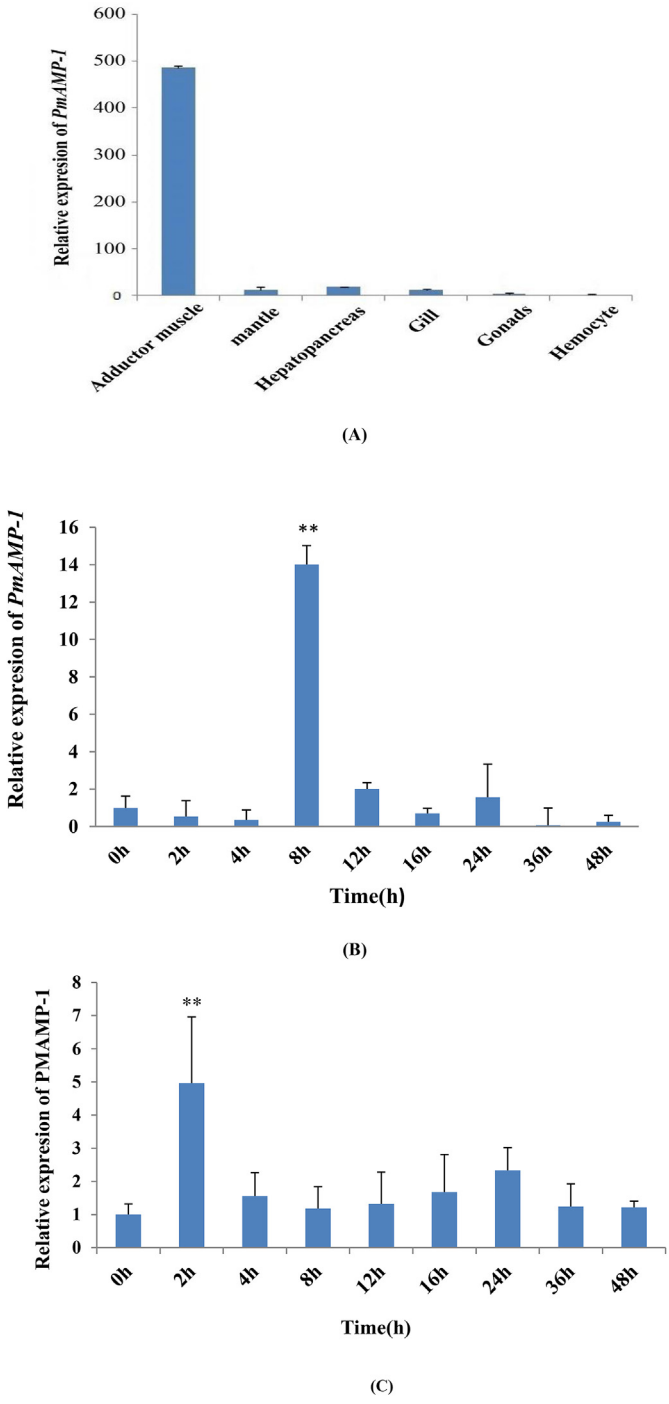

Fig. 6. Quantitative analysis of *PmAMP-1* expression. (A) Tissue-specific expressionofPmAMP-1. Vertical bars represent the mean ± SD of three technical replicates. TheY axis of each graph is scaled based on the highest level of expression. (B) Temporal expression of PmAMP-1in hemocytes after challenge. (C) Temporal expression of PmAMP-1in adductor muscle after challenge.Significant differences between control and challenge groups are indicated with twoasterisk at $P < 0.01$. Vertical bars represent themean ± SD ($n = 3$).

The structural predictions suggest that the secondary structure of PmAMP-1 likely to be made up of random coils, extended strands, and α-helices. Nine hydrophobic amino acids (positions 82–90) are clustered near the C-terminus, and are the main amino acids forming a C-terminal random coil, and the five hydrophobic amino acids near the N-terminus (positions 39–44) form an alpha helix. These structural

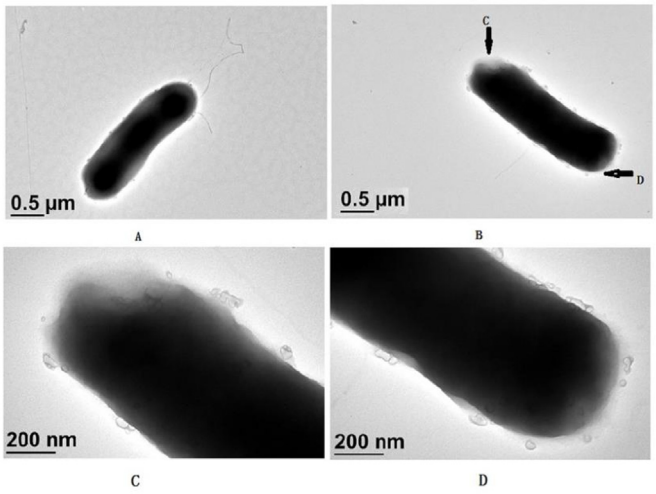

Fig. 7. Transmission Electron Microscopy of *E. coli*untreated and treated with PmAMP-1.A representcontrol*E. coli*;B represent*E. coli*treated with PmAMP-1; C and D are enlarged images of arrows showed in B.

characteristics may have a role to play in PmAMP-1's antimicrobial function and the prediction that it has an unordered, non-amphipathic structure suggests that this peptide might not be able to directly perturb/target lipid bilayers and may exert its antimicrobial effect by a mechanism other than the formation of toroidal pores [38].

The predicted tertiary structure of PmAMP-1 is characterized mainly by the presence of several α-helices and random coils, and an absence of beta-sheets. The online prediction of the PmAMP-1 structure revealed that no functional domains nor signal peptides are present and that it is considered to be a new protein with antibacterial activity. Because of the fact that the sequence of PmAMP-1 did not match any available models nor similar sequences for modeling, the predicted structure of PmAMP-1 presented here requires further evidence for confirmation.

The expression patterns of *PmAMP-1* were investigated to better understand its physiological functions. Under normal conditions, *PmAMP-1* was constitutively expressed in all examined tissues, though transcript levels varied in different tissues. This indicates that *PmAMP-1* plays a key role as a ubiquitously expressed factor in *P.f. Martensii* and is involved in a wide array of cellular processes. The expression pattern of PmAMP-1 was very similar to that described for mytichitin A in *Mytilus coruscus*; higher levels in the adductor muscle and the lowest expression levels in hemocytes [39]. Similarly, another AMP cgRPL29 from the Pacific oyster (*Crassostrea gigas*) is also highly expressed in the adductor muscle [40].

To further understand the possible biological function of PmAMP-1 in the stress response, mRNA expression was examined at different time points after bacterial challenge. In the present study, we used hemocytes as they are primarily responsible for defense against pathogens in bivalves [41]. As a main component of the immune response in pearl oysters, hemocytes are believed to be functionally analogous to vertebrate leukocytes and, therefore, play a crucial role in the recognition and removal of foreign materials [42]. In this study, *PmAMP-1* transcription was clearly time dependent in hemocytes after bacterial challenge. The highest levels of *PmAMP-1* expression occurred 8 h after bacterial challenge, indicating that PmAMP-1 is an immune protein. This was in good agreement with the expression pattern of other AMPs under bacterial challenge in *Ruditapes philippinarum* [43] and *Hyriopsis cumingii* [44].

Because PmAMP-1 is highly expressed in the adductor muscle, the temporal expression of PmAMP-1 was determined in the adductor muscle upon bacterial challenge. The transcription of *PmAMP*-1 mRNA was upregulated at 2 h (4.96-fold, $P < 0.01$), with a 4.96-fold increase

when compared to the control group in this study. After a peak at 2 h, the expression level went back to normal over the following hours. These results reveal that *P.f. Martensii* might respond rapidly to invading bacteria by synthesizing AMPs that participate in host immune defense. The temporal expression pattern of the *PmAMP-1* gene in the adductor muscle after challenge further contributes to our overall understanding of acute-phase proteins in anti-pathogenic responses.

The PmAMP-1 identified in this study was found to significantly inhibit the growth of *E. coli* and *M. luteus*, which is consistent with the results of the AMPs identified from other molluscs. Antimicrobial peptides in *Mytilus*, including Mytilin and Myticin, appear to exhibit broad-spectrum antibacterial activity [18–20].

Current research shows that the antibacterial peptides act on bacteria in two ways: one is via membrane lysis; while the other is not based on membrane lysis [45]. However, most AMPs exert antibacterial activity by acting on bacterial cell membranes or cell walls [45,46]. For example, studies have shown that Magainin and Melitin inhibit the synthesis of target cell wall components through complex mechanisms that kill target cells, while some antimicrobial peptides can kill bacteria directly [47,48]. As shown in this study, PmAMP-1 can make the cell wall and membrane collapse at both ends in *E. coli*, initiating the release of substances from the bacteria to form a vesicular structure attached to its surface. The mechanism of action of PmAMP-1 appears to be membrane lysis and begins from both ends of the cell. This mechanism is different from the action of other AMPs. For example, myticusin-1 from *M. coruscus* triggered morphological alteration in *E. coli,* including the formation of mesosome-like structures, cell wall effects and the separation of cytoplasmic membrane from cell wall [24]. Flesh fly element II inhibited the growth of bacteria by inhibiting the formation of the cell wall, making the bacteria unable to maintain normal cellular morphology and causing cell wall perforation [49]. Although the exact mechanism of action of PmAMP-1 on bacteria has not been resolved, the evidence reported here indicates that PmAMP-1 has a unique mechanism of action.

Compared with other studies on terrestrial biological antimicrobial peptides, such as those from insects, relatively few antimicrobial peptides isolated and purified from marine organisms have been reported [2,50]. Marine biological antimicrobial peptides have received increasing attention due to their special evolutionary status and the particularities of the environment in which marine organisms exist [51–53]. This experiment provides new information for immunological research and disease control in *P.f. Martensii*.

Acknowledgments

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fsi.2019.07.002.

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