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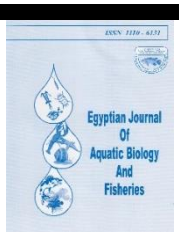

Molecular Identification and Hemocyanin Gene (HMC) Characterization of the Shrimp *Litopenaeus vannamei* Infected by Acute Hepatopancreatic Necrosis Disease (AHPND)

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ABSTRACT

Hemocyanin is a crucial non-specific innate immune defense molecule, exhibiting phenoloxidase, antiviral, antibacterial, hemolytic, and antitumor activities. In this study, the expression profile of hemocyanin (HMC) from the shrimp *L. vannamei* was isolated and characterized to better understand the mechanisms of functional diversity, particularly in recognizing various pathogens and enhancing immune responses. The study focused on identifying HMC gene characteristics from *L. vannamei* to serve as a source of resistance genes in shrimp. The research involved sampling *L. vannamei* from Gresik Regency and Situbondo Regency in East Java, Indonesia. RNA isolation from the hepatopancreatic organs was achieved, followed by reverse transcription to obtain cDNA. This was followed by cDNA amplification through PCR and subsequent electrophoresis. The results indicated strong HMC expression in healthy shrimp compared to those infected with AHPND. These data confirm that the HMC gene plays a significant role in the resistance of *L. vannamei*.

INTRODUCTION

The shrimp farming industry, particularly *Litopenaeus vannamei*, has experienced rapid annual growth in Indonesia **(Anwar *et al.*, 2024; Pramudia *et al.*, 2024)**. The obstacle experienced is the problem of disease that often cannot be avoided even though shrimp fry has been well selected. The primary disease affecting white shrimp is acute hepatopancreatic necrosis disease (AHPND) **(Lozano-Olvera *et al.*, 2024)**. The results of

monitoring the genetic character of the white shrimp mothers imported from Hawaii and Florida showed low gene diversity (homozygous). This is understandable because this white shrimp mother is also the result of selective breeding from its country of origin, which is already the eighth derivative (F-8). At the same time, it is assumed to be F-0 in Indonesia. The results of monitoring the derived fry vary significantly in terms of growth, disease resistance, and resilience to environmental infections. This variability is attributed to selective breeding practices, which can lead to the loss of other important trait-control genes in the process. Consequently, problems arise in the offspring produced, including growth delays, increased susceptibility to viral infections, and vulnerability to environmental stressors.

When extreme ecological changes occur that are unsuitable for the organism's regular life needs, physiological processes will adjust in response to gene control mechanisms. Continuous exposure to unsuitable environmental conditions can lead to genetic changes as the organisms attempt to adapt.

Among the resistance-coding genes in shrimp is hemocyanin. Hemocyanin is a multifunctional protein primarily found in the hemolymph of arthropods and molluscs, which has recently been reported to play an essential role in oxygen transport, moulting regulation, and non-specific immune defence of antigens **(Coates & Nairn, 2014)**. Based on research, it was deduced that hemocyanin from shrimp *L. vannamei* has antigenic, agglutinative, hemolytic, and immunity-enhancing activities. However, so far, information regarding the molecular basis underlying the multifunction of hemocyanin has not been available **(Lu *et al.*, 2015)**. It has been reported that HMC can functionally be converted into various forms, including antimicrobial peptides **(Yan *et al.*, 2011)**, antiviral agents **(Wyles *et al.*, 2011)**, antifungal compounds **(Liu *et al.*, 2018)**, agglutinin and other proteins **(Zhan *et al.*, 2019; Bao *et al.*, 2020; Monteiro *et al.*, 2020; Aweya *et al.*, 2021; Yang *et al.*, 2021)**.

In addition, other studies have shown that HMC from the shrimp *L. vannamei* can act as a protein that increases immunity **(Qiao *et al.*, 2011; Cao *et al.*, 2014)**. These results suggest that HMC is vital in many immune and innate immune system activities in invertebrates. But so far, little is known about the molecular mechanisms of HMC functional diversity in *L. vannamei*.

For this reason, research is needed on identifying and characterizing hemocyanin from *L. vannamei* to track the source of resistance genes in shrimp. In this study, we explored hemocyanin (HMC) to be isolated and represented through a proteomic approach to HMC diversity in *L. vannamei* at the gene level. In future studies, the relationship between HMC diversity at the protein level and resistance to pathogens will be analyzed, as well as the characterization of the mechanism. These findings will help to understand the multifunctional molecular basis of HMC and establish new strategies for disease control of *L. vannamei.*

MATERIALS AND METHODS

Sampling

The white shrimp (*L. vannamei*) specimens were collected from two locations: Gresik Regency and Situbondo Regency in East Java, Indonesia. The shrimp selected were 40 days old post-fry stocking (DOC age of 40). A total of 90 white shrimps were taken for the study. Samples were then stored at -80°C to preserve the hepatopancreas in stable condition **(Niemcharoen *et al.*, 2022)**.

Molecular identification of shrimp is suspected to be AHPND

Molecular detection of acute hepatopancreatic necrosis disease (AHPND) was performed using the PCR method for diagnosing *nterocytozoon hepatopenaei* (EHP) **(Ma *et al.*, 2021)**. Microsporidia isolates were extracted from the white shrimp exhibiting signs of hepatopancreatic microsporidia disease. Hepatopancreas samples from the shrimp were processed using lysis buffer kits **(Lozano-Olvera *et al.*, 2024)**.

The amplification process included an initial denaturation cycle at 94°C for 3 minutes, followed by 35 cycles of denaturation at 94°C for 30 seconds, annealing at 60°C for 30 seconds, and extension at 72°C for 30 seconds. A final extension was performed at 72°C for 5 minutes. The results of the PCR reaction were subjected to electrophoresis for 30 minutes at a voltage of 70 volts and were subsequently visualized using a UV transilluminator.

Identification and profile characters of hemocyanin gene (HMC)

RNA isolation

Isolation of RNA was performed using Qiagen's QIAzol® Lysis Reagent kit **(de Souza Valente *et al.*, 2020)**. The procedure adapts to the RNA isolation procedure handbook with the QIAzol® Lysis Reagent kit. A hepatopancreas sample was taken and weighed to 0.1-0.2 grams. The hepatopancreas sample was then inserted into a 2ml tube and labelled based on each sample name. 500µl of qiazole was added and chopped using scissors and a micro pestle until the hepatopancreas was destroyed and dissolved, and then another 500µl of qiazole was used. The sample was then incubated at room temperature for 5 minutes. Then 200µl chloroform was added and homogenized with vortex until it foamed cloudy. The sample was then incubated at room temperature for 3 minutes and then centrifuged at a rate of 12,000 x g for 15 minutes at 4ºC. The function of centrifugation is to separate the particulate components of a sample to obtain a supernatant **(Ghanaati *et al.*, 2019)**.

After completion of the centrifugation process, interphase samples were taken and then transferred to a new tube measuring 1.5ml. The selection was added with 500µl of isopropanol for RNA precipitation **(Carapito *et al.*, 2023)** and then turned slowly. Next, the sample was incubated at room temperature for 10 minutes, and then centrifuged at 12,000 x g for 10 minutes at 4ºC. After the second centrifugation, the pellets were collected from the sample tube, and 1,000 µl of 75% ethanol (EtOH) was added for preservation **(Johnson *et al.*, 2023)**. The mixture was then centrifuged at 7,500 x g for 5 minutes at 4ºC.

Following this, the pellets were dried in a laminar air flow (LAF) hood with the light on and the blower turned off, for approximately 1.5 hours. The pellets were then dissolved in 50µl of nuclease-free water (NFW), and an aliquot of 10µl was transferred to a thin-walled tube. The RNA isolation results were stored in a freezer at -20°C.

To assess the quality of the RNA isolation, a 1% agarose gel electrophoresis was conducted using ethidium bromide (EtBr) for staining **(Motohashi, 2019)**. Electrophoresis was performed at 50 volts for 55 minutes. The quantity of RNA was measured using a nanodrop spectrophotometer at A260 and A280nm wavelengths **(Li *et al.*, 2020)**. The absorbance ratio at A260/A280 indicates the purity of the RNA sample. RNA concentration was calculated by multiplying the absorbance at 260nm by a constant and the dilution factor (A260 x 50 x 1). Good RNA purity ranges from 1.7 to 2.0 **(Groven *et al.*, 2023)**, allowing for subsequent reverse transcription to obtain cDNA.

Reverse transcription

Reverse transcription is the process of converting RNA into complementary DNA (cDNA) **(Sessegolo *et al.*, 2019)**. This process is essential for characterizing gene expression. The reverse transcription was conducted using the ReverTra Ace™ qPCR RT Master Mix with gDNA remover from Toyobo.

The first step involved preparing a reagent by mixing 29.4µl of 4x DNA-MM with 0.6µl of gDNA remover. RNA samples were then incubated at 65°C and placed on ice. Next, a new reagent was prepared, consisting of 2µl of the first reagent, 5µl of nuclease-free water, and 1 µl of RNA template. The sample was incubated again at 37°C.

Following this, 5x RT Master Mix II was added, and the incubation proceeded at the following temperatures: 27°C for 15 minutes, 50°C for 5 minutes, and 98°C for 5 minutes. After this process, the quantity of cDNA was tested using the same method as for RNA. Good DNA purity ranges from 1.7 to 2.0 **(Sophian, 2021)**.

Amplification of cDNA

The amplification of cDNA aims to double the amount of cDNA. The amplification process includes several key steps: hot start, denaturation, annealing, extension, and post-extension **(Obande & Banga Singh, 2020)**.

The hot start prepares the thermal cycler for amplification, performed at 95°C for 2 minutes. During denaturation, the double-stranded DNA is separated into single strands, carried out at 95°C for 30 seconds. The next step, annealing, involves the attachment of primers to the cDNA template created during denaturation; this occurs at 59°C for 45 seconds.

The extension phase lengthens the DNA strands at 72°C for 30 seconds, followed by a post-extension step at the same temperature for 5 minutes. The total number of cycles used in this process is 32.

The first step in the cDNA amplification is to prepare a PCR mix, the composition of which is detailed in Table (1). A diluted cDNA sample is then added to achieve a final concentration of 50ng/ µl, with 1µl of template included. The sample is placed into the thermal cycler, and the program is set for amplification. The composition of the PCR mix is shown in Table (2).

For this research, the primers used are hemocyanin_1673R21 as the reverse primer (5'-GGG ATC ATG GGT GGC AGT TTC-3') and hemocyanin_115F24 as the forward primer (5'-CAT ATG GAA TTC CCM TTC TGG TGG-3'). These are specific primers that bind to the nucleotide sequences coding for the hemocyanin gene.

Unlike universal primers, specific primers attach to particular nucleotide sequences needed for profiling and gene expression. The forward primer contains an M base and a substitution base. Base substitutions in primers involve changes in the nucleotide sequence that occur as a non-complementary break, replacing the original sequence **(Oka *et al.*, 1994)**.

Electrophoresis

The electrophoresis process functions to separate cellular molecules based on their size, using an electric field that is flowed in a medium containing samples to be separated **(Xin *et al.*, 2019)**. Electrophoresis was performed with a 1.5% agarose medium. The electrophoresis process was carried out by running 50 volts for 55 minutes. The results of electrophoresis were seen on the UV transilluminator.

RESULTS

1. Molecular analysis

The testing results using this PCR method showed that the white shrimp cultivated were detected by disease. Pools 1, 2, and 3 showed positive PCR results infected with EHP (Fig. 1)

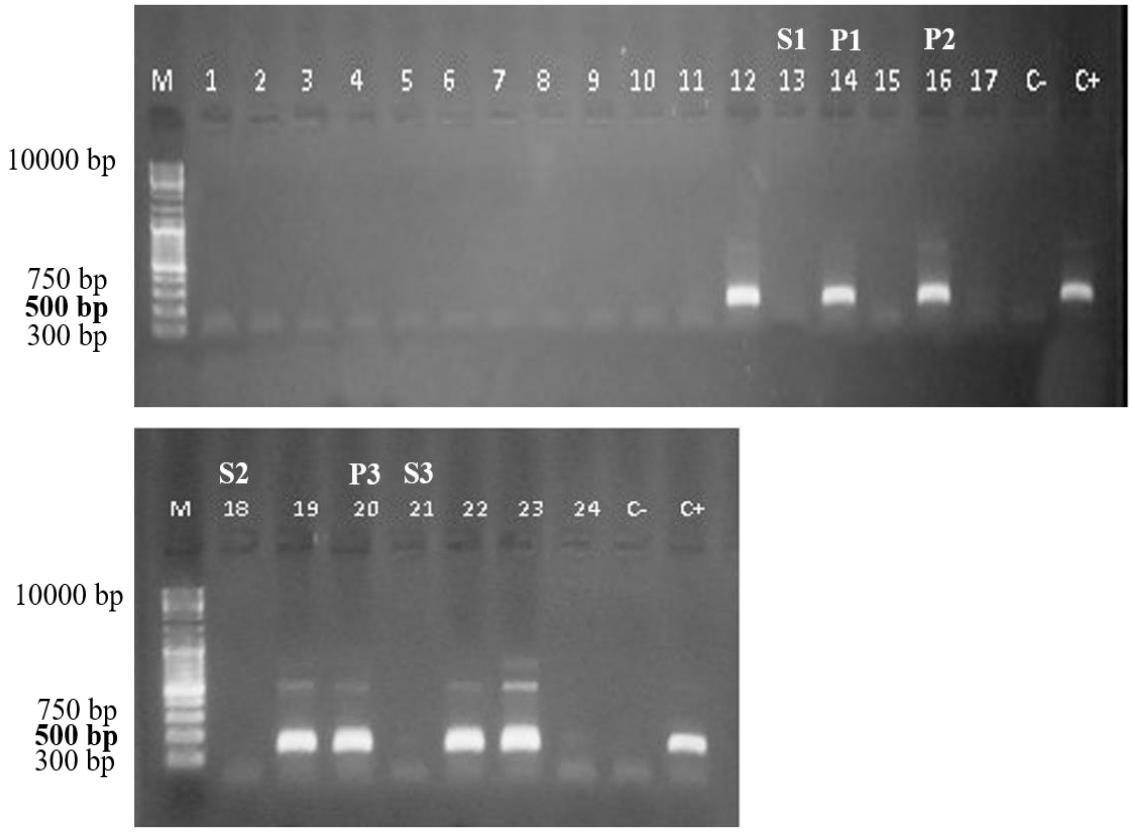

Fig. 1. Molecular analysis of white shrimp infected by AHPND

PCR results from P1 have marker number 14, P2 has marker number 16, and P3 has marker number 20, indicating positive PCR results for EHP. A positive EHP result can be seen in a DNA marker line parallel to the positive control and have thick and firm band. The sample was found to have a line parallel to the positive control, most likely a positive sample of EHP. The sample found does not have a line parallel to the positive control, thus the sample is expected to be harmful to AHPND.

2. RNA Isolation

The results of RNA quality and quantity tests provide insights into the RNA isolation process. The RNA quantity test results, obtained using a nanodrop spectrophotometer, are presented in Table (4). These results indicate both the concentration and purity of the RNA.

RNA samples with a purity value between 1.7 and 2.0 are suitable for the subsequent reverse transcription process. The RNA quality test results are illustrated in Fig. (2).

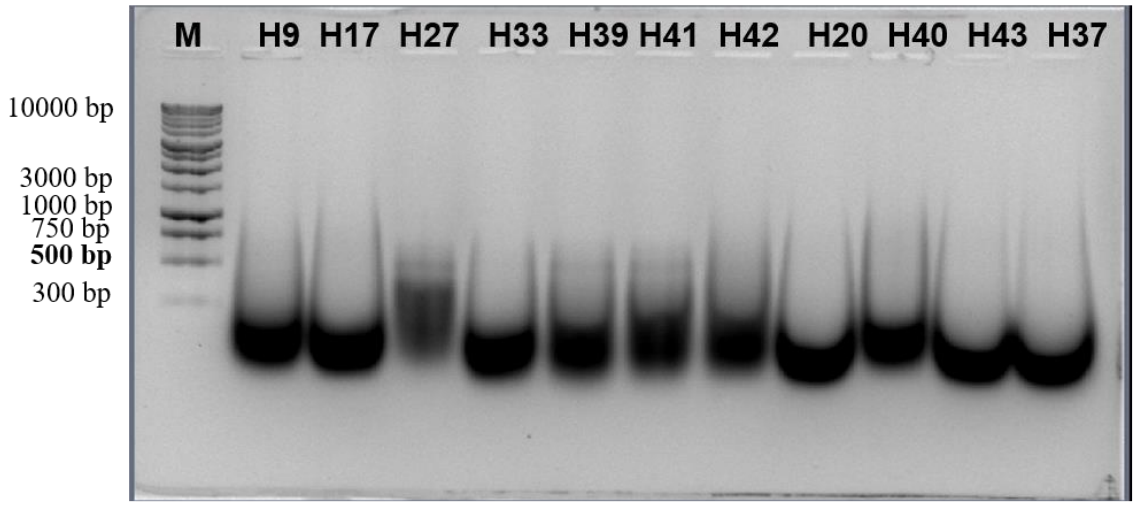

Fig. 2. RNA quality and quantity tests of sample

3. Reverse transcription result

The results of the cDNA quantity test are shown in Table (1). There is cDNA with a value below 1.7 as much as 1 sample, while ten other samples have cDNA purity with a range of 1.7-2.0.

Table 1. cDNA of health shrimp (H) and AHPND infected shrimp (S) during the research

No	Sample	cDNA of health shrimp (H) and AHPND infected shrimp (S)	
		Concentration (ng/ul)	Purity (A260/280)
1	H2	2014.6	1.739
2	H3	2813.8	1.336
3	H5	2232.4	1.723
4	H6	2168.5	1.703
5	H9	1834.3	1.73
6	H17	1780.2	1.731
7	H27	1717.6	1.741
8	H33	1839.1	1.745
9	H39	1632.9	1.743
10	H41	1734.6	1.739
11	H42	1751.4	1.74
12	SA6	2321.6	1.7
13	SA7	2010.3	1.728
14	SA12	1444.7	1.765
15	SA15	1705.4	1.752
16	SA16	2069.4	1.714
17	SB2	28.91	1.71
18	SB7	12.04	1.7
19	SC2	1588.8	1.764
20	SC5	1860.9	1.734
21	SC14	1393.6	1.765

4. PCR result

The results of the PCR process are visualized with the GelDoc UV transilluminator, as shown in Figs. (3, 4). Based on PCR results in healthy shrimp and shrimp infected with AHPND, the results showed that bands in healthy shrimp appear thick and firm. Whereas in shrimp infected with AHPND, bands do not appear.

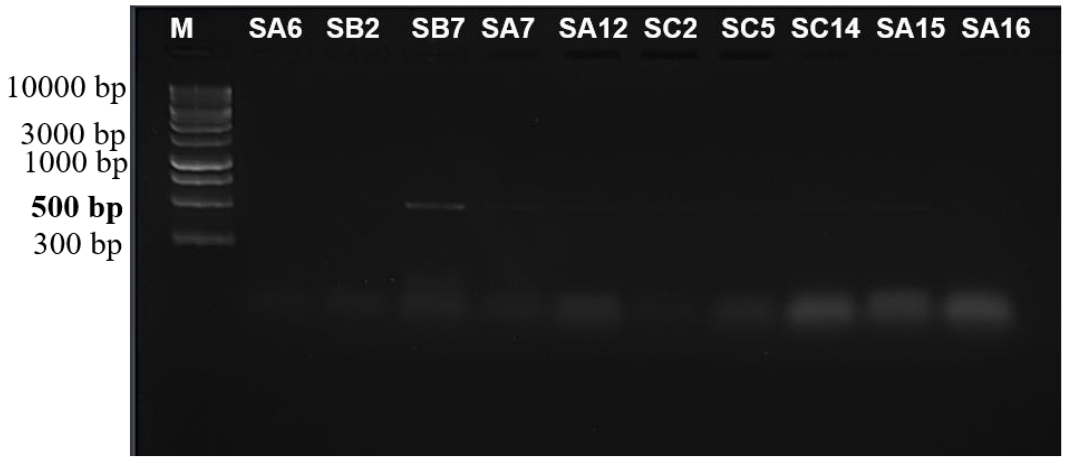

Fig. 3. AHPND shrimp PCR result

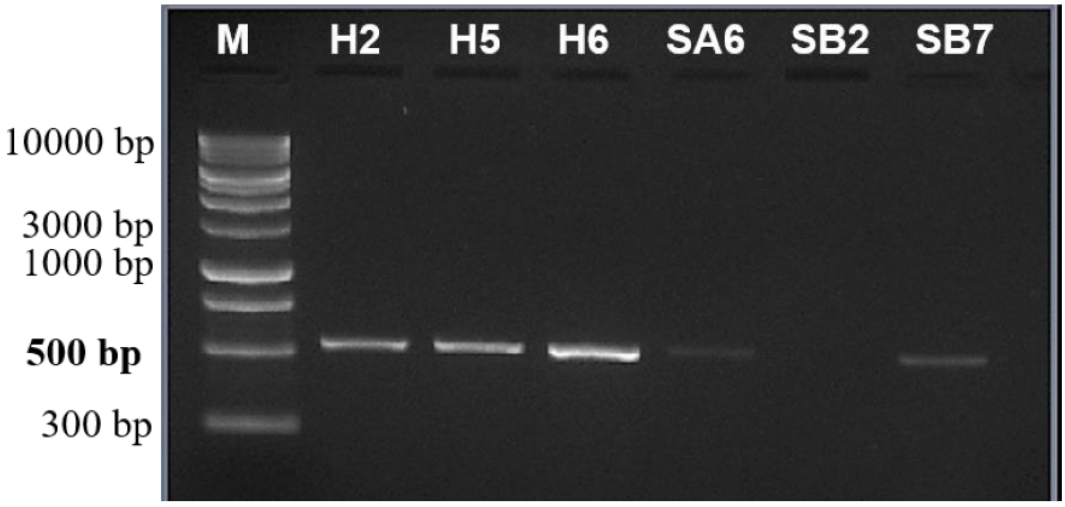

Fig. 4. Health shrimp PCR result (H)

DISCUSSION

The study of the hemocyanin gene (HMC) in *L. vannamei* infected with acute hepatopancreatic necrosis disease (AHPND) is crucial for understanding the molecular mechanisms underlying shrimp immunity and disease resistance. Hemocyanin, a multifunctional protein, is primarily recognized for its role in oxygen transport in arthropods and mollusks including shrimp. Recent studies have expanded its functional repertoire, highlighting its involvement in immune responses and moulting regulation,

which are vital for shrimp's resilience against pathogens **(Aguilera-Rivera *et al.*, 2018; Aweya *et al.*, 2022)**.

The characterization of the hemocyanin gene expression profile is essential for elucidating its functional diversity and potential role in disease resistance, particularly against AHPND, which poses significant threats to shrimp aquaculture **(Hùng *et al.*, 2021; Zhang *et al.*, 2021)**. The research aimed to isolate and characterize the expression profile of the hemocyanin gene, hypothesizing that it could serve as a genetic marker for resistance to AHPND. This gene was chosen due to its established significance in innate immune defense mechanisms, suggesting that enhanced understanding and manipulation of hemocyanin could lead to improved disease resistance in shrimp populations **(Aguilera-Rivera *et al.*, 2018; Zhang *et al.*, 2021)**. The study's findings indicated that healthy shrimp exhibited strong hemocyanin expression, while infected individuals showed diminished or absent expression, reinforcing the potential of hemocyanin as a marker for shrimp health and a target for selective breeding programs aimed at enhancing disease resistance **(Hùng *et al.*, 2021; Zhang *et al.*, 2021)**.

In conducting this research, samples were meticulously collected from shrimp farms in Gresik and Situbondo Regencies in Indonesia. The shrimp samples were at a developmental stage of 40 days post-fry stocking, a critical period for assessing health and disease susceptibility **(Hùng *et al.*, 2021; Zhang *et al.*, 2021)**. The samples were preserved at -80°C to maintain the integrity of the hepatopancreas, which was subsequently analyzed for signs of AHPND. Molecular identification techniques, including RNA isolation, reverse transcription to cDNA, and polymerase chain reaction (PCR), were employed to detect the presence of the *Enterocytozoon hepatopenaei* (EHP) pathogen, frequently associated with AHPND **(Hùng *et al.*, 2021; Zhang *et al.*, 2021; Aweya *et al.*, 2022)**.

The PCR results, visualized through electrophoresis, demonstrated a clear correlation between hemocyanin expression levels and shrimp health status, with healthy shrimp showing robust bands indicative of successful gene expression **(Hùng *et al.*, 2021; Zhang *et al.*, 2021)**. The meticulous RNA isolation procedure involved homogenizing hepatopancreas samples with Qiazole lysis reagent, followed by chloroform separation and isopropanol precipitation to ensure high-quality RNA for downstream applications. The purity of RNA samples was critical for accurate results, with only those meeting the purity ratio criteria proceeding to reverse transcription **(Hùng *et al.*, 2021; Zhang *et al.*, 2021; Aweya *et al.*, 2022)**.

The subsequent PCR amplification utilized specific primers for the hemocyanin gene, allowing for precise assessment of gene expression in both healthy and infected shrimp. The results underscored the potential of hemocyanin as a genetic marker for disease resistance, as its expression was significantly higher in healthy shrimp compared to those affected by AHPND **(Hùng *et al.*, 2021; Zhang *et al.*, 2021)**. In conclusion, the study successfully characterized the hemocyanin gene in *L. vannamei*, elucidating its role in disease resistance and laying the groundwork for future research aimed at improving

shrimp health through genetic interventions. The findings suggest that manipulating hemocyanin expression could lead to the development of superior shrimp broodstock with enhanced resistance to AHPND, thereby contributing to the sustainability of the shrimp farming industry **(Hùng *et al.*, 2021; Zhang *et al.*, 2021)**.

CONCLUSION

The hypothesis that the HMC gene codes for body resistance in shrimp is supported by the research results. The findings indicated that healthy shrimp displayed a thick and well-defined band, suggesting successful binding of the primer to the hemocyanin gene. In contrast, shrimp infected with AHPND showed no bands, indicating that the primer did not successfully bind to the hemocyanin gene. This absence of bands is likely due to the inactivity of the hemocyanin gene in AHPND-infected shrimp, resulting in reduced or absent expression.

The results suggest that the HMC gene in healthy shrimp is robust, indicating that the resistance gene is functional. Conversely, in shrimp infected with AHPND, the HMC gene, which represents the resistance gene, is not active.

Looking ahead, it is hoped that the HMC gene can be utilized as a source of resistance genes, potentially serving as an insertion agent to produce superior, disease-resistant shrimp broodstock.

REFERENCES

Aguilera-Rivera, D.; Escalante-Herrera, K.; Gaxiola, G.; Prieto-Davó, A.; Rodríguez-Fuentes, G.; Guerra-Castro, E.; Hernández-López, J.; Chávez-Sánchez, M.C. and Rodríguez-Canul, R. (2018). Immune response of the pacific white shrimp, litopenaeus vannamei, previously reared in biofloc and after an infection assay with vibrio harveyi. J. World Aquac. Soc., 50(1): 119-136. https://doi.org/10.1111/jwas.12543

Anwar, A.; Zainuddin; Djawad, M. I.; Aslamyah, S.; Saade, E. ; Karim, M. Y.; Trijuno, D. D. ; Murni ;Syafiuddin; Mohammed, A. A. and Imam Taukhid, I. (2024). Effect of Soybean Meal Substitution Using Raintree (Samanea saman) Seed Meal on the Physical Quality of Feed and Growth Performance of the Juvenile White Shrimp, Litopenaeus vannamei. Egyptian Journal of Aquatic Biology & Fisheries, 28(4): 2249 – 2274

Arslan, M.; Tezcan, E.; Camci, H. and Avci, M.K. (2021). Effect of DNA Concentration on Band Intensity and Resolution in Agarose Gel Elec-trophoresis. Van Sağlık Bilimleri Dergisi., 14(3): 326–333. https://doi.org/10.52976/vansaglik.969547

Aweya, J.J.; Zheng, Z.; Zheng, X.; Yao, D. and Zhang, Y. (2021). The expanding repertoire of immune-related molecules with antimicrobial activity in penaeid shrimps: a review. Rev. Aquac., 13(4): 1907-1937. https://doi.org/10.1111/raq.12551

Bao, S.; Zheng, Z.; Aweya, J.J.; Yao, D.; Li, S.; Sun, C.; Hong, Y. and Zhang, Y. (2020). microRNA-589-5p modulates the expression of hemocyanin as part of the anti-WSSV immune response in Litopenaeus vannamei. Dev. Comp. Immunol., 107: 103642 https://doi.org/10.1016/j.dci.2020.103642

Cao, J.; Wang, Z.; Zhang, Y.; Qu, F.; Guo, L.; Zhong, M.; Li, S.; Zou, H.; Chen, J. and Wang, X. (2014). Identification and characterization of the related immune-enhancing proteins in crab Scylla paramamosain stimulated with rhubarb polysaccharides. Mol. Immunol., 57(2): 263-273. https://doi.org/10.1016/j.molimm.2013.10.003

Carapito, R.; Bernardo, S.C.; Pereira, M.M.; Neves, M.C.; Freire, M.G. and Sousa, F. (2023). Multimodal ionic liquid-based chromatographic supports for an effective RNA purification. Sep. Purif. Technol., 315: 123676. https://doi.org/10.1016/j.seppur.2023.123676

Coates, C.J. and Nairn, J. (2014). Diverse immune functions of hemocyanins. Dev. Comp. Immunol., 45(1): 43-55. https://doi.org/10.1016/j.dci.2014.01.021

de Souza Valente, C.; Oliveira Ortiz, K.; Depperschmidt, R.; de Medeiros Fraga, A. P.; do Nascimento Vieira, F. and Freire Marques, M.R. (2020). Transcription of defense related genes in Pacific white shrimp, Litopenaeus vannamei, kept in biofloc and in clear seawater and challenged with the white spot syndrome virus. Aquac. Int., 28(1): 293–307. https://doi.org/10.1007/s10499-019-00461-8

Ghanaati, S.; Mourão, C.; Adam, E.; Sader, R.; Zadeh, H. and Al-Maawi, S. (2019). The role of centrifugation process in the preparation of therapeutic blood concentrates: Standardization of the protocols to improve reproducibility. Int. J. Growth Factors Stem Cells Dent., 2(3): 41. https://doi.org/10.4103/gfsc.gfsc_15_19

Groven, R.V.M.; Blokhuis, J.T.; Poeze, M.; van Griensven, M. and Blokhuis, T.J. (2023). Surgical suction filter-derived bone graft displays osteogenic miRNA and mRNA patterns. Eur. J. Trauma Emerg. Surg., 50(1): 315-326. https://doi.org/10.1007/s00068-023-02350-5

Hùng, N.; Aranguren, L.; Cruz-Flores, R.; White, B. and Dhar, A. (2021). Differentially expressed genes in hepatopancreas of acute hepatopancreatic necrosis disease tolerant and susceptible shrimp (penaeus vannamei). Front. immunol., 12. https://doi.org/10.3389/fimmu.2021.634152

Johnson, G.; Canty, S.W.J.; Lichter-Marck, I.H.; Wagner, W and Wen, J. (2023). Ethanol preservation and pretreatments facilitate quality DNA extractions in recalcitrant plant species. Appl. Plant Sci., 11(3). https://doi.org/10.1002/aps3.11519

Li, Y.; Zhou, F.; Huang, J.; Yang, L.; Jiang, S.; Yang, Q. and Jiang, S. (2020). Transcriptome and miRNA profiles of black tiger shrimp, Penaeus monodon, under different salinity conditions. Front. Mar. Sci., 7: 579381.

Liu, S.; Aweya, J.; Zheng, L.; Wang, F.; Zheng, Z.; Zhong, M.; Lun, J. and Zhang, Y. (2018). A Litopenaeus vannamei Hemocyanin-Derived Antimicrobial Peptide (Peptide B11) Attenuates Cancer Cells' Proliferation. Mol., 23(12): 3202. https://doi.org/10.3390/molecules23123202

Lozano-Olvera, R.; Abad-Rosales, S.M.; Soto-Rodriguez, S.A. and Aguilar-Rendón, K.G. (2024). Time course of acute hepatopancreatic necrosis disease (AHPND) in the Pacific white shrimp Penaeus vannamei by wet mount analysis. Aquaculture International, 32(3): 2313-2329.

Lu, X.; Lu, H.; Guo, L.; Zhang, Z.; Zhao, X.; Zhong, M.; Li, S. and Zhang, Y. (2015). Cloning and characterization of a novel hemocyanin variant LvHMCV4 from shrimp Litopenaeus vannamei. Fish Shellfish Immunol., 46(2): 398-405. https://doi.org/10.1016/j.fsi.2015.06.022

Ma, C.; Fan, S.; Wang, Y.; Yang, H.; Qiao, Y.; Jiang, G.; Lyu, M.; Dong, J.; Shen, H. and Gao, S. (2021). Rapid detection of Enterocytozoon hepatopenaei infection in shrimp with a real-time isothermal recombinase polymerase amplification assay. Front. cell. infect. microbiol., 11: 631960.

Mazlan, A.H., Muhamad, N.M.H.A.; Hazizul H.M.; Mohd H.F.H. and Mohd Y.R. (2024). Effect of DNA template concentration on standard polymerase chain reaction. International Journal of Pharmaceutical, Nutraceutical and Cosmetic Science (IJPNaCS), 7(1): 1-11.

Monteiro, M.L.; Lima, D.B.; Menezes, R.R.P.P.B.; de, S.T.L.; Silva, B.P.; Serra N.J.V.; Cavalcanti, M.M., Morlighem, J.E. and Martins, A.M.C. (2020). Antichagasic effect of hemocyanin derived from antimicrobial peptides of penaeus monodon shrimp. Exp. Parasitol., 215: 107930. https://doi.org/10.1016/j.exppara.2020.107930

Motohashi, K. (2019). Development of highly sensitive and low-cost DNA agarose gel electrophoresis detection systems, and evaluation of non-mutagenic and loading dye-type DNA-staining reagents. PLoS ONE., 14(9). https://doi.org/10.1371/journal.pone.0222209

Niemcharoen, S.; Haetrakul, T.; Palić, D. and Chansue, N. (2022). Microplastic-Contaminated Feed Interferes with Antioxidant Enzyme and Lysozyme Gene Expression of Pacific White Shrimp (Litopenaeus vannamei) Leading to Hepatopancreas Damage and Increased Mortality. Animals., 12(23). https://doi.org/10.3390/ani12233308

Obande, G.A. and Banga S.K.K. (2020). Current and future perspectives on isothermal nucleic acid amplification technologies for diagnosing infections. Infect. Drug. Resist., 12(13): 455-483. https://doi.org/10.2147/idr.s217571

Oka, T.; Matsunaga, H.; Tokunaga, K.; Mitsunaga, S.; Juji, T. and Yamane, A. (1994). A simple method for detecting single base substitutions and its application to HLA-DPB1 typing. Nucleic. Acids. Res., 22(9): 1541–1547. https://doi.org/10.1093/nar/22.9.1541

Pramudia, Z.; Kurniawan, A.; Prayogo, T.B.; Dwi, Y.A.; Susanti, S.; Al Zamzami, I.M. and Moehammad, K.S. (2024). Profitability analysis and sensitivity level of white shrimp (Litopenaeus vannamei) cultivation using the millennial shrimp farming (msf) ECSOFiM (Economic and Social of Fisheries and Marine Journal), 11(2): 155-165.

Qiao, J.; Du, Z.; Zhang, Y.; Du, H.; Guo, L.; Zhong, M.; Cao, J. and Wang, X. (2011). Proteomic identification of the related immune-enhancing proteins in shrimp Litopenaeus vannamei stimulated with vitamin C and Chinese herbs. Fish. Shellfish. Immunol., 31(6): 736-745. https://doi.org/10.1016/j.fsi.2011.07.005

Sessegolo, C.; Cruaud, C.; Da Silva, C.; Cologne, A.; Dubarry, M.; Derrien, T.; Lacroix, V. and Aury, J.M. (2019). Transcriptome profiling of mouse samples using nanopore sequencing of cDNA and RNA molecules. Sci. Rep., 9(1): 14908. https://doi.org/10.1038/s41598-019-51470-9

Sophian, A. (2021). DNA Isolation of Chicken Feathers from the Base of the Young Feathers, the Base of the Old Feathers, and the Tip of the Feathers. Bioeduscience, 5(2): 104-108.

Wyles, D.L.; Jones, K.A.; Valiaeva, N.; Beadle, J.R.; Schooley, R.T. and Hostetler, K.Y. (2011). Enhanced Cellular Penetration of ODE-(S)-MPMPA Accounts for Its Prolonged Post-exposure Anti-HCV Activity. Antiviral Research, 90(2): A65-A66. https://doi.org/10.1016/j.antiviral.2011.03.133

Xin, A.; Zhang, R.; Yu, K. and Wang, Q. (2019). Mechanics of electrophoresis-induced reversible hydrogel adhesion. J. Mech. Phys. Solids., 125: 1-21. https://doi.org/10.1016/j.jmps.2018.12.007

Yan, F.; Zhang, Y.; Jiang, R.; Zhong, M.; Hu, Z.; Du, H.; Lun, J.; Chen, J. and Li, Y. (2011). Identification and agglutination properties of hemocyanin from the mud crab (Scylla serrata). Fish Shellfish Immunol., 30(1): 354-360. https://doi.org/10.1016/j.fsi.2010.11.004

Yang, S.; Huang, H.; Aweya, J.J.; Zheng, Z.; Liu, G. and Zhang, Y. (2021). PvHS9 is a novel in silico predicted antimicrobial peptide derived from hemocyanin of Penaeus vannamei. Aquac., 530. https://doi.org/10.1016/j.aquaculture.2020.735926

Zhan, S.; Aweya, J.J.; Wang, F.; Yao, D.; Zhong, M.; Chen, J.; Li, S. and Zhang, Y. (2019). Litopenaeus vannamei attenuates white spot syndrome virus replication by specific antiviral peptides generated from hemocyanin. Developmental and Comparative Immunology, 91: 50-61. https://doi.org/10.1016/j.dci.2018.10.005

Zhang, Q.; Yu, Y.; Luo, Z.; Xiang, J. and Li, F. (2021). Comparison of gene expression between resistant and susceptible families against vpahpnd and identification of biomarkers used for resistance evaluation in litopenaeus vannamei. Front. Genet., 12. https://doi.org/10.3389/fgene.2021.772442