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# Clone and expression of the LvCTL3 gene encoding C-type lectin from *Litopenaeus vannamei* shrimp and its agglutination ability of AHPND–causing *Vibrio parahaemolyticus*

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## Abstract

C-type lectins (CTL) are a family of carbohydrate-binding proteins which it not only recognize and bind to potentially pathogenic microbes but also activate the complement pathway in the innate immunity of invertebrates. In this study, LvCTL3 protein was bonded by cloning the LvCTL3, which was isolated from *Litopenaeus vannamei* shrimp into the pET200/D-TOPO vector to construct a recombinant pET200/LvCTL3 plasmid. The plasmid was then transferred into competent *E. coli* BL21 (DE3) cells to express the protein. The expression of LvCTL3 protein was confirmed by SDS-PAGE. Pathogen binding assays confirmed recombinant LvCTL3 protein had strong agglutination with the pathogenic *Vibrio parahaemolyticus,* which caused acute hepatopancreas necrosis (AHPND) in white-leg shrimp. This preliminary study showed LvCTL3 protein acted as a PRR that inhibited AHPND-causing bacteria and could be used for

**Keywords:** *Agglutination, C-type lectin, expression, Litopenaeus vannamei, Vibrio parahaemolyticus*

## Introduction

White-leg shrimp *Litopenaeus vannamei* (Boone, 1931) plays an important role in the economy development of Southeast Asian countries, and in the Americas, producing worth billions of dollars (Shekhar et al. 2021). However, the rapid expansion of intensive shrimp culture leads to severe disease outbreaks, which has become a significant challenge in commercial shrimp culture. In recent years, the spread of these diseases has caused significant economic loss to shrimp farmers (Phuong et al., 2023b). Phuong et al, (2023a) indicated that a causative agent of acute hepatopancreatic necrosis disease (AHPND), caused mass mortality for shrimp in Vietnam. Unlike intenerates, shrimp lack adaptive immune systems and only have innate immune responses that provide a broad and effective response to the invading pathogenic microorganisms (Roy et al., 2020). These defence systems consist of pattern recognition triggering a series of immune responses (Wang et al., 2020; Phuong et al., 2023b). Lectins are proteins/glycoproteins capable of reversible binding via non-covalent bonds to carbohydrates, without altering their structure. Lectins can bind to cells with glycoproteins or microbial surface glycolipids and are considered PRRs for the recognition and binding to invading microorganisms. The shrimp lectin protein family is rich and diverse; C-type lectin (CTL) is the most varied and well-studied lectin (Zhang et al., 2009). Various types of CTL with structural and functional diversity may constitute a recognition network against invading bacterial and viral pathogens and play essential roles in the defense system of shrimp (Li & Xiang, 2013; Viana & Maggioni, 2022). I Viana & Maggioni, (2022) described the lectins of *L. vannamei* are divided three types: C-type lectin, L-type lectin and galectin, which are mainly expressed in the hepatopancreas and hemocytes. They are involved in several immune response pathways, including phagocytosis, hemocyte recruitment, prophenoloxidase activation, and gene regulation. After synthesis in the hepatopancreas, these CTLs are released into the blood, and stored within granular cells (Junkunlo et al., 2012). In previous studies, two CTL genes from *L. vannamei* (*LvCTL*$_3$ and *LvCTL*$_4$) have been cloned, sequenced, and characterized. The encoding sequence of the *LvCTL3* gene was 444 nucleotides, and the deduced polypeptide sequence had 147 amino acids. In contrast, the *LvCTL*$_4$ gene had a length of 417 nucleotides, and the deduced polypeptide sequence had 138 amino acids (Phuong et al., 2023b). The expression level of *LvCTL*$_3$ was significantly higher in the hepatopancreas of *L. vannamei* infected with *Vibrio*. Meanwhile, the expression level of LvCTL3 tended to increase in the intestine, stomach, and hemocytes when infected with *F. solani*, causing black gill symptoms in shrimp. This suggested that LvCTL3 plays

a role in the innate responses that resist pathogen invasion (Phuong et al., 2023c). The aim of this study was to clone and express the LvCTL3 gene from *L. vannamei* and to evaluated the agglutination ability of this recombinant LvCTL3 on AHPND-causing *Vibrio parahaemolyticus*.

## Materials and Methods

### Materials

The recombinant p-GEM T-easy vector containing the *LvCTL3* gene (pGEM/CTL3) was obtained in a previous report (Phuong et al., 2023b). The AHPND-causing Vibrio parahaemolyticus TTHVP202101001 strain, which was isolated from white-leg shrimp culture ponds in Phong Dien district, Thua Thien Hue province, Vietnam, was obtained from the Laboratory of Fish Pathology, University of Agriculture and Forestry, Hue city, Vietnam (Phuong et al., 2023a).

### Methods

#### *Cloning LvCTL3 into expression vector*

The recombinant pGEM/CTL3 vector was used as a template in PCR amplification with specific primers (Table 1). The PCR component consisted of 1 Unit Phusion$^{TM}$ High-Fidelity DNA Polymerase (ThermoFisher Scientific, USA), 10 µL 5× Phusion$^{TM}$ HF Buffer, 1 µL 10 mM dNTPs, 20 pmol each primer, 1.5 µL DMSO, 3.6 µL of vector, and distilled water to a final volume of 50 µL. PCR was performed using the following conditions: 98°C for 30 s, 35 cycles of 98°C for 10 s, 51°C for 30 s, and 72°C for 30 s, followed by a final extension at 72°C for 7 min (SimpliAmp Thermal Cycler, Applied Biosystems, USA). PCR products were confirmed by electrophoresis at 70V for 35 min in a 1% agarose gel with TAE 1X stained SafeViewTM Classic (ABM, Canada, Cat#: G108).

The PCR products were purified using the GeneJET Gel Extraction Kit (Thermo Fisher Scientific, USA). Purified PCR products were cloned into the pET200/D-TOPO vector harboring the T7 promoter, ribosome-binding site, initiation ATG, polyhistidine (6xHis) region, two TOPO® recognition sites, and a T7 transcription termination region (Invitrogen, USA). Composition of the ligation reaction was comprised of 1 µL vector, 1 µL salt solution, 2 µL PCR product (8 ng), and 2 µL distilled water, which were mixed well and incubated at 23C for 5 min. The recombinant vectors were transferred into competent *Escherichia coli* TOP10 cells using the heat-shock method. Competent cells were subcultured in 200 µL SOC medium (2% tryptone,

0,5% yeast extract, 10 mM NaCl, 2,5 mM KCl, 10 mM $MgCl_2$, 10 mM $MgSO_4$, and 20 mM glucose, pH = 7). Then, 800 µL of LB medium (1% tryptone, 0,5% yeast extract, 1% NaCl) was added, and the culture was shaken at 37°C for 60 min. Cells were subcultured on solid LB medium supplemented with 100 µg/mL kanamycin at 37°C overnight, and recombinant cells were selected by PCR with specific LvCTL3 primers.

**Table 1.** Specific primers for coding sequence of *LvCTL3* gene

| **Primers** | **Sequences** |
|---|---|
| LvCTL3 | Forward: 5'- **CACC**TCTCCAATCCCATCTCAATC -3' |
| | Reverse: 5'- CTATTTCTCACAGATAATG - 3' |
| T7 | Forward 5'- TAATACGACTCACTATAGGG -3' |
| | Reverse: 5'- TAGTTATTGCTCAGCGGTGG -3' |
| M13 | Forward: 5'- GTAAAACGACGGCCAGTG -3' |
| | Reverse: 5'- GGAAACAGCTATGACCATG-3' |

***** To enable directional cloning in the further, the forward primers contain the CACC sequence (adapter) at the 5'end. These four nucleotides will base pair with the GTCC overhang sequence in pET200/D-TOPO vector (5741 bp, Invitrogen).

*The cells of* E. coli carrying the recombinant vector were then inoculated with 5 mL of LB (Luria Bertani, Himedia, India) broth supplemented with 100 µg/mL Kanamycin, and then DNA plasmids were isolated using the GeneJET Plasmid Miniprep Kit (ThermoFisher Scientific, USA). Recombinant vectors were then transformed into competent *E. coli* BL21 Star™ (DE3) cells (3 µL ligation solution + 50 µL cells + 250 µL SOC medium) by Champion™ pET200 Directional TOPO® Expression Kit (Invitrogen) according to the manufacture protocol. After 30 min of transformation at 37°C, the solution was put into 5 mL of LB medium containing 100 µg/mL Kanamycin and overnight incubated at 37°C, 200 rpm until an $OD_{600}$ of approximately 1.0 was obtained.

***Expression of the LvCTL3 gene in E. coli***

The $OD_{600}$ of approximately 1.0 of *E. coli* BL21 Star™ (DE3) cells containing the recombinant vector with the coding sequence of LvCTL3 gene was harvested. Then, isopropyl β-D-thiogalactopyranoside (IPTG) was added to a final concentration of 0.8 mM for induction of expression recombinant protein of the *LvCTL3* gene for 6 h at 28°C. The cells were collected by centrifugation at 13000 rpm for 10 min. Cell pellet were lysed by sonication at 60 Hz for 30 min

(QSONICA Ultrasonic Processor, USA), and the soluble protein was dissolved in TNE extraction buffer (50 mM Tris. HCl (pH 7.5), 100 mM NaCl, and 2 mM EDTA) supplemented with 1% Triton X-100 and 1.0 µg/mL Lysozyme. Total soluble protein was concentrated by centrifuging at 13000 rpm at 4°C for 15 min. The insoluble residues were resolved in 8M Urea solution and the inclusion body protein was obtained again by centrifuging (Thuy et al., 2021).

Expression of LvCTL3 was checked by electrophoresis on a 12% (w/v) polyacrylamide gel. The gel was then stained with Coomassie Blue R-250 for 1 h with 45 rpm and discoloration for 2 h to detect protein samples, and the image was analyzed with a protein marker 10-180 kDa (ThermoFisher Scientific, USA).

***Preparation of recombinant LvCTL3***

The recombinant BL21 cell was dissolved in 5 mL lysis buffer (20 mM Tris-HCl, 5 mM EDTA, 1 M NaCl, pH 8.0), vortexed, and incubated on ice for 60 mins. Cells were broken by sonication at 60 Hz for 30 min (QSONICA Ultrasonic Processor, USA), and protein (soluble and insoluble) was obtained by centrifuging at 13000 rpm at 4°C for 15 min.

The inclusion body of proteins was washed in Triton X-100 buffer (20 mM Tris-HCl, 2% Triton X-100, pH 8.0) 5 times and washed again once with free Triton X-100 buffer (20 mM Tris-HCl, pH 8.0), and then dissolved in the buffer (20 mM Tris-HCl, 4 M Guanidine HCl, pH = 8.0). The solution was incubated in ice for 1 hour (with shaking), and centrifuged at 13000 rpm for 10 mins to collect the dissolved and precipitated phase for SDS analysis. The soluble and insoluble fraction phase were refolded by rapid dilution at 4ºC in the refolding solution (20 mM Tris-HCl, 2 M Urea, 10% Sucrose, 0,1% Tween-80, pH 8.0), collecting the soluble phase and precipitated phase in the refolding solution (Nhat et al, 2019).

***Vibrio parahaemolyticus agglutinating assay***

*Vibrio parahaemolyticus* TTHVP202101001 was cultured in 5 mL of Tryptone Soya Broth medium supplemented with 2% NaCl (TSB, HiMedia, India) at 28°C with shaking at 200 rpm for 24 hours. The bacterial broth suspension was centrifuged at 13,000 rpm for 10 mins and the cell pellet was washed twice by 0.85% sterile saline. The cell pellet was re-suspended and adjusted to give an optical density ($OD_{600}$) value of 1 using TBS- $Ca^{2+}$ buffer (50 mM Tris-HCl, 100 mM NaCl, 10 mM $CaCl_2$, pH = 7.5) (equivalent to $10^8$ CFU/mL), and then 10-fold serial dilutions performed to give approximately 1 x $10^6$ CFU/mL for the pathogen binding study.

The *Vibrio parahaemolyticus* agglutinating assay was performed in 96 wells plastic plate using five treatments with 50 µL of *Vibrio parahaemolyticus* at $10^6$ CFU/mL and in treatments 1-5, the cell suspension was add with different ratio of the recombinant LvCTL3 as followed: (i) Treatment 1 (T1 - control): only *Vibrio parahaemolyticus* without the recombinant LvCTL3 added; (ii) Treatment 2 (T2): 50 µL of *Vibrio parahaemolyticus* in TBS without $Ca^{2+}$ buffer and 50 µL of LvCTL3 (1:1); (iii) Treatment 3 (T3): 50 µL of *Vibrio parahaemolyticus* in TBS- $Ca^{2+}$ buffer and 25 µL LvCTL3 (ratio 1:0.5); (iv) Treatment 4 (T4): 50 µL of *Vibrio parahaemolyticus* in TBS- $Ca^{2+}$ buffer and 50 µL LvCTL3 (1:1), and Treatment 5 (T5) : 50 µL of *Vibrio parahaemolyticus* in TBS- $Ca^{2+}$ buffer and 100 µL LvCTL3 (1:2). Each treatment was conducted in triplicates and all treatments were incubated at 25°C for 02 h. The reaction mixture was then grown in TSB medium (2% NaCl) at 28°C for 24 h. The growth ability of *V. parahaemolyticus* was determined at 600nm and the *V. parahaemolyticus* binding activity was confirmed under the light microscope (Thao et al., 2020).

***Statistical analysis***

The data of the *Vibrio parahaemolyticus* agglutinating assay were expressed as mean ± SD, and analyzed by one-way ANOVA followed by a LSD test, using SPSS software (Version 16.0) to evaluate whether the means were significantly different ($P < 0.05$).

## Results

### Cloning *LvCTL3* gene into pET200/D-TOPO vector

The pGEM/CTL3 vector was used as template for amplifying the *LvCTL3* gene. The *LvCTL3* gene on the pGEM/CTL3 vector was successfully amplified without contamination because of the absence of any DNA band on agarose gel in negative control (from pET200/LvCTL3 vector in *E. coli* TOP10 with LvCTL3 primers*)*. There was only one band about 450 bp when using LvCTL3 primers and one band about 600 bp when using M13 primer (Figure 1). The PCR product with the size of 450 bp was selected for purify. Purified PCR products were cloned into pET200/D-TOPO vector and transformed into *E. coli* TOP10 cells. After growing overnight on solid LB agar with kanamycin, transformed colonies were confirmed by direct PCR with LvCLT3 and T7 primers (Figure 1). Thus, *the LvCTL3 gene was successfully cloned into the pET200/D-TOPO vector; the recombinant expression vector was named* pET200/LvCTL3. In this study, the *LvCTL3* gene was successfully recovered by PCR using the Phusion™ HighFidelity DNA Polymerase enzyme

(ThermoFisher Scientific, USA). This enzyme amplifies highquality products, does not create by-products, and makes the initial product used in the TOPO binding reaction. The pET200/LvCTL3 vector was isolated from recombinant cells, then successfully transformed into competent *E. coli* BL21(DE3) cells, five transformed colony and confirmed by colony PCR (Fig.2), there was only one band of about 450 bp when using *LvCLT3* primer and one band of about 650 bp when using the T7 primer (Fig. 2).

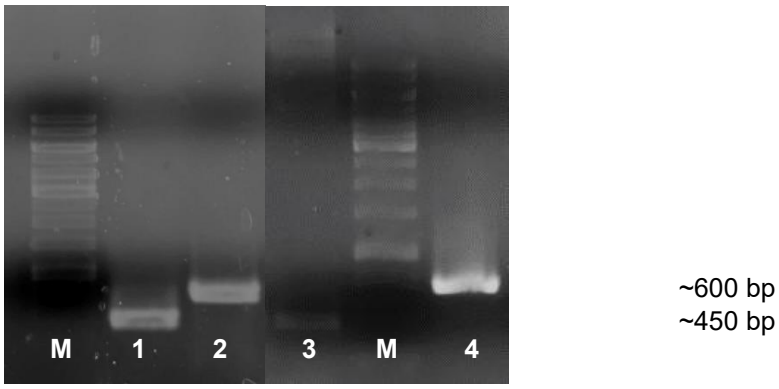


**Figure 1.** Cloning of *LvCTL3* into expression vector

*M. DNA standard size (GeneRuler$^{TM}$ 1 kb DNA Ladder, ThermoFisher Scientific, USA); 1: PCR product from pGEM/CLT3 vector with LvCTL3 primers, 2. PCR product from pGEM/CLT3 vector with M13 primers. 3. PCR product from pET200/LvCTL3 vector in E. coli TOP10 with LvCTL3 primers. 4. PCR product from pET200/LvCTL3 vector in E. coli TOP10 with T7 primers*.

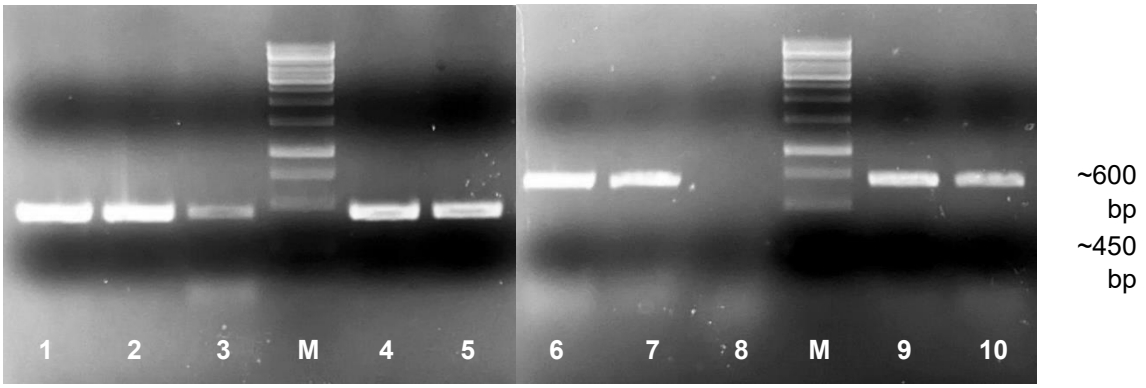


**Figure 2.** PCR products from pET200/LvCTL3 vector in *E. coli* BL21(DE3) colonies

*M. DNA standard size (GeneRuler$^{TM}$ 1 kb DNA Ladder, ThermoFisher Scientific, USA). 1-5: PCR product with LvCTL3 primers, 6-10. PCR product with T7 primers.*

**Expression of *LvCTL3* gene in *E. coli***

The *E. coli* BL21 Star™ (DE3) cells containing the pET200/D-TOPO vector with the coding sequence of the *LvCTL3* gene were cultured in LB medium supplemented with 0.8 mM IPTG to determine the production of LvCTL3 can be produced. Four colonies carrying the recombinant vector pET200/LvCTL3 (Figure 2) were selected for evaluation of their ability to express recombinant LvCTL3. As analyzed by polyacrylamide gel electrophoresis of the soluble and inclusion forms (Figure 3), an extra protein band with a molecular weight of approximately 20 kDa was observed in the inclusion form in transformed *E. coli* cells. This protein was absent in *E. coli* cells treated with the control and soluble extracts. Thus, it can be seen that a protein with a molecular weight corresponding to LvCTL3 appears in *E. coli* BL21 cells. In particular, LvCTL3 has a predicted size of 16.3 kDa (corresponding to 444 bp, 148 amino acids), and the 6xHis fusion fragment has a size of 3.7 kDa.

After solubilization, the LvCTL3 protein was refolded using a rapid dilution method. The results showed that a protein band of 20 kDa appeared in the protein sample after refolding (Figure 4). This proved that the LvCTL3 protein was successfully refolded. Thus, after the refolding process, the LvCTL3 protein from the inclusions was collected in a soluble form. The results showed that the LvCTL3 protein was successfully refolded by a simple process that existed in a soluble form and could continue to test its agglutination ability.

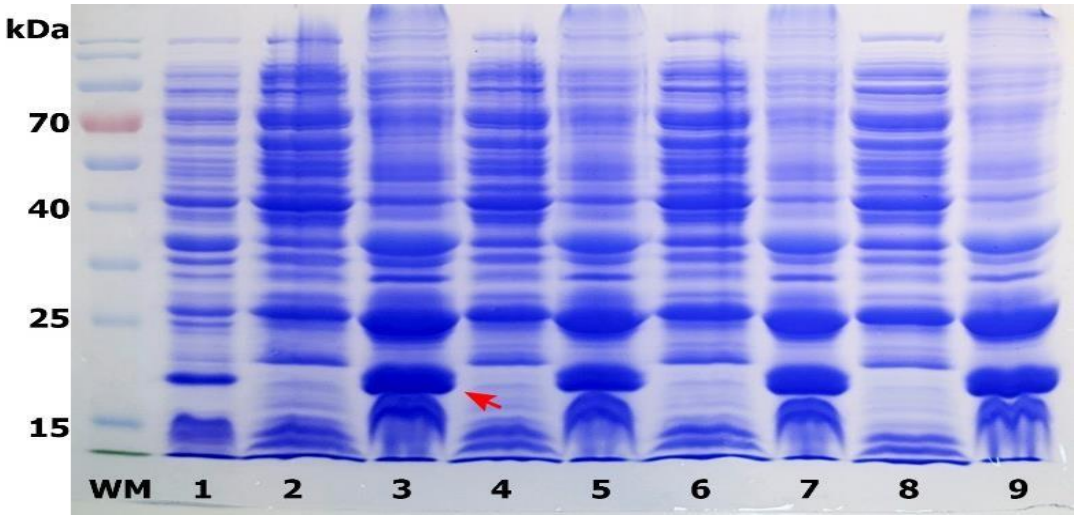


**Figure 3.** Polyacrylamide gel electrophoresis of total protein from *E. coli* cells

*WM: Protein marker (ThermoFisher Scientific, USA). 1: inclusion of control (transformed E. coli cells without induction of IPTG), 2; 4; 6; 8: soluble protein form of 4 colonies; 3; 5; 7; 9: inclusion protein form of 4 colonies.*

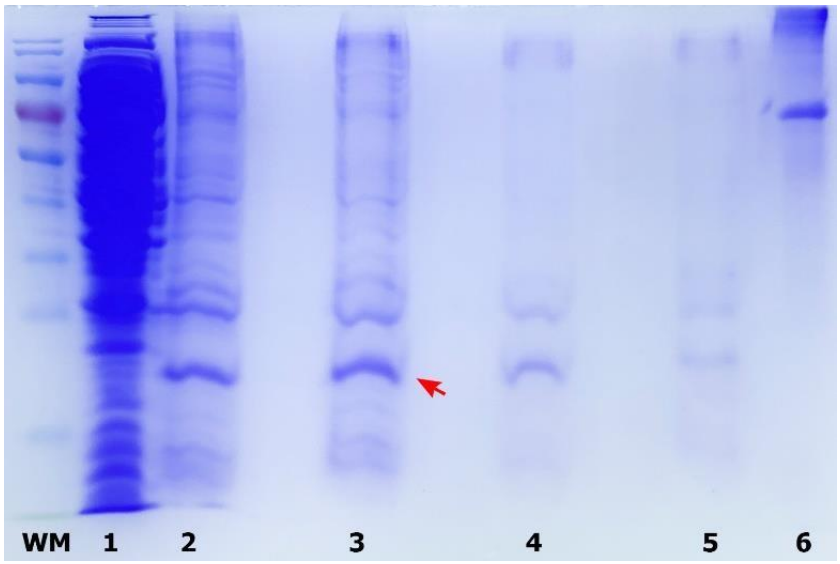


**Figure 4.** Polyacrylamide gel electrophoresis of refolding protein from inclusion body

*WM: Protein marker (ThermoFisher Scientific, USA). 1. Soluble protein; 2-5: refolding protein. 6. Stardard BSA (500 µg/mL).*

**Agglutination activity of recombinant LvCTL3**

The recombinant LvCTL3 from the inclusion body in *E.coli* was refolded and applied to *Vibrio parahaemolyticus* TTHVP202101001 agglutination. The growth of *V. parahaemolyticus* was significantly inhibited when co-cultured with LvCTL3. The cell density ($OD_{600}$) of control (without recombinant LvCTL3) was twice higher than that in the treatment with recombinant LvCTL3 added at the ration of 1:1 (Table 1). Under the light microscope, *V. parahaemolyticus* bacteria was agglutinated by recombinant LvCTL3 protein in TBS-Ca buffer (Fig. 5). These results supported the hypothesis that recombinant LvCTL3 was able to agglutinate AHPNDcausing *V. parahaemolyticus* in the Ca supplemented medium.

**Table 1.** *Vibrio parahaemolyticus* agglutination activity of recombinant LvCTL3

| No | Treatments | $OD_{600}$ |
|---|---|---|
| **T1** | *Vibrio* (Control) | $1.984^{a} \pm 0.020$ |
| **T2** | *Vibrio*: LvCTL3 (ratio 1:1) | $0.971^{c} \pm 0.003$ |
| **T3** | *Vibrio* + $Ca^{2+}$ : LvCTL3 (ratio 1:0.5) | $1.467^{b} \pm 0.012$ |
| **T4** | *Vibrio* + $Ca^{2+}$ : LvCTL3 (ratio 1:1) | $0.865^{d} \pm 0.020$ |
| **T5** | *Vibrio* + $Ca^{2+}$ : LvCTL3 (ratio 1:2) | $0.665^{e} \pm 0.002$ |

*Data presented as Mean ± SD of three replicates per treatment. The $^{a, b, c, d, e}$ with different superscript letters on the same column for statistical differences ($P < 0.05$).*

The density of *V. parahaemolyticus* bacterial in the control treatment (without LvCTL3 and $Ca^{2+}$) reached 1.984, which is higher than the other treatments ($P < 0.05$). For treatment T2 without the presence of $Ca^{2+}$, the density of *V. parahaemolyticus* reached 0.971 which was significantly higher than all treatments with presence of $Ca^{2+}$ ($P < 0.05$). This results showed that the *V. parahaemolyticus* agglutination activity of recombinant LvCTL3 depends on presence of $Ca^{2+}$ which was in line with the study of Li et al., (2014). Specifically, this study indicated that, in treatments with the presence of $Ca^{2+}$, the higher ratio of recombinant LvCTL3 the more agglutination of *V. parahaemolyticus* was observed. Specifically the treantment 5 (T5), the value of $OD_{600}$ was only 0.665 which was significantly lower than other treatments ($P < 0.05$).

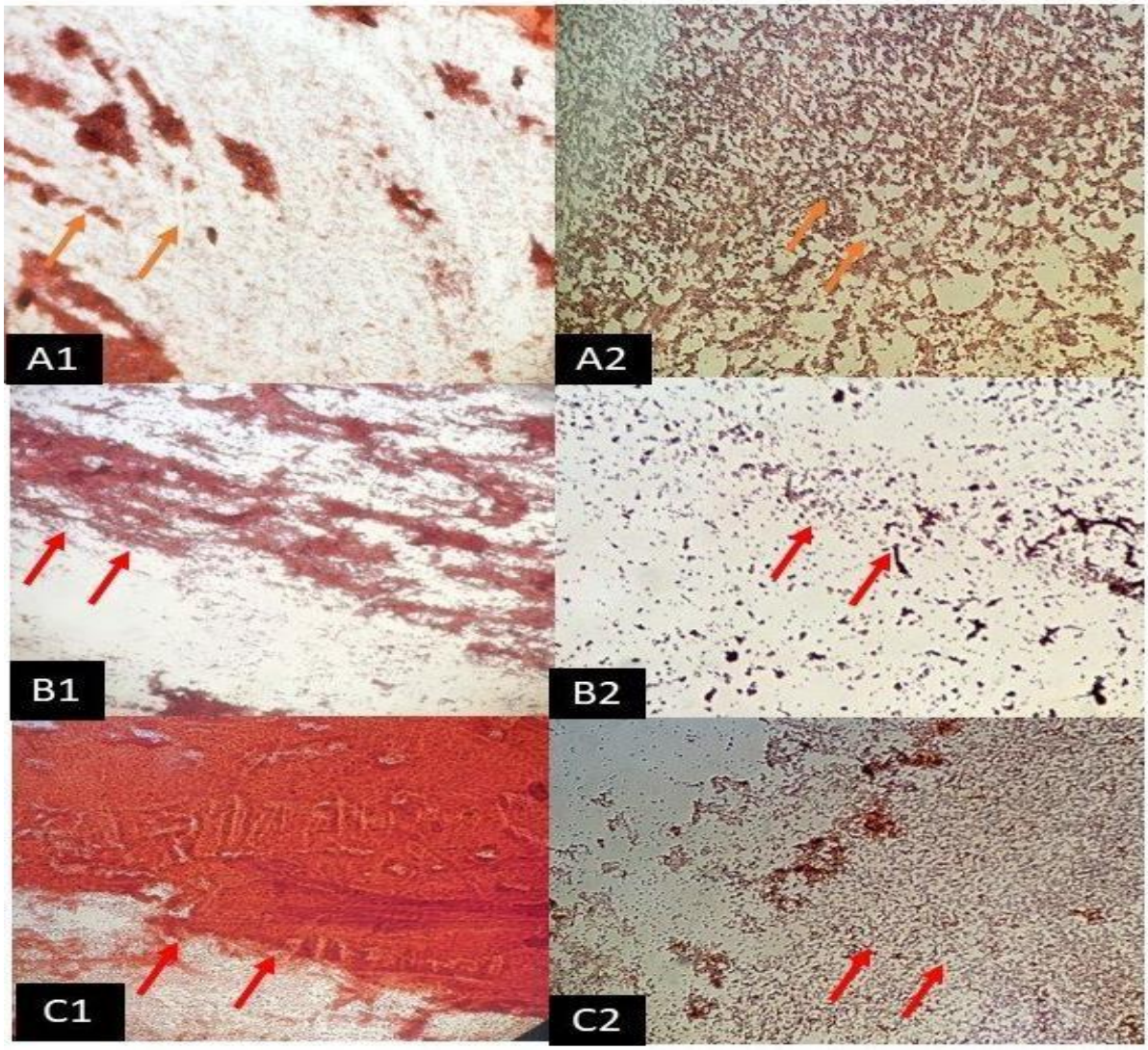


**Figure 5.** *Vibrio parahaemolyticus* TTHVP202101001 agglutination ability of recombinant LvCTL3 (magnification: 10× (A1-C1) and 40× (A2-C2) respectively)

*A1-A2 control (without LvCTL3), B1-B2. V. parahaemolyticus treated with LvCTL3 (1:1), C1C2. V. parahaemolyticus treated with LvCTL3 and $Ca^{2+}$ (1:1).*

## Discussions

Immune responses are essential to protect organisms from invading pathogens. Like other invertebrates, shrimp induce humoral and cellular immune responses when exposed to bacteria or pathogen-associated molecular patterns (PAMPs). These proteins play crucial roles in pathogen identification and neutralization. Most lectins associated with the immune response in shrimp show specific expression or exhibit the highest expression levels in the hepatopancreas, such as LvLT, LvCTL1, and LvCTL5. Currently, there are seven types of lectins are found in shrimp: Ctype, L-type, P-type, M-type, fibrinogen-like domain, galectins, and calnexin/calreticulin. Inwhichs, CTL are the most researched and interested. CTLs belong to a large superfamily, and are widely distributed in almost all organisms. They are characterized by $Ca^{2+}$-dependent ligand recognition through a C-type carbohydrate recognition domain (CRD), which plays a role in innate immune responses, such as pattern recognition receptors and agglutination, and contributes to increased antibacterial and antiviral activities in arthropods by recognizing and binding to specific CTL domains (Luo et al., 2019; Ma et al, 2007; Zhao et al., 2009).

The immune function of a NLR-like gene in crustaceans was firstly described by Li et al., (2022). The breakdown of LvNLRPL1 has resulted to accelerating the proliferation of *Vibrio* in the hepatopancreas and increased the mortality rate of shrimps after *Vibrio* infection. Runsaeng et al., (2015) reported a new C-type lectin, designated FmLC1, was cloned from the hepatopancreas of the banana shrimp (*Fenneropenaeus merguiensis*) when shrimp was challenged with *Vibrio harveyi* or white spot syndrome virus. Other studies also identified that two CTLs (*LvLectin-1* and *LvLectin-2*), as well as an upregulation of the *LvCTL3* gene were highly expressed in the hepatopancreas of white-leg shrimp during experimentally challenge with *Vibrio anguillarum* (Wang & Wang, 2013). In this study, the *LvCTL3* gene from shrimp (*L. vannamei*) has a length of 579 bp, in which the coding segment is 492 bp (from nucleotide positions 25 to 516), encoding a polypeptide chain with a length of 163 amino acids. *LvCTL3* may play significant role in innate immunity of shrimp against bacterial and viral infections. The prevoius study showed that the purified LvCTL3 might agglutinate Gram-negative microbe *Vibrio alginolyticus* and *V. parahaemolyticus* and Gram-positive bacteria *Bacillus subtilis* in the presence of $Ca^{2+}$, but was not be able to agglutinate *Streptococcus agalactiae* Gram-positive bacteria. In addition, *LvCTL3* mRNA can be detected in all tested tissues and recombinant LvCTL3 protein can agglutinate both pathogenic Gram-negative and Gram-positive bacteria in shrimp, and significantly reduce the

mortalyti of *V. parahaemolyticus* and WSSV infection (Li et al., 2014). In the previous reported, we isolated a *LvCTL3* fragment from *L. vannamei*, it was 444 bp in size (including the stop codon) and there was 98.87% homologous to the published gene (439/444 nucleotides compared with KF156943). Analysis results showed that the change in the *LvCTL3* gene in a difference in the sequence of the encoded polypeptide, with the highest similarity obtained at 97.28% (143/147 amino acids) compared to the reference sequence AGV68681. Using bioinformatics tools, the function of predicted LvCTL3 had no difference (Phuong et al., 2023b). The expression of *LvCTL3* in tissues revealed an increasing trend in the intestine, stomach, and hemocytes following AHPND infection. Additionally, a strong expression of *LvCTL3* was observed in the hepatopancreatics of AHPND-infected shrimps. This finding highlights the significant role of the *LvCTL3* gene in the shrimp defense system, as the upregulation of the CTL-encoding gene in key body parts during disease infection suggests its involvement in protection against invading pathogens (Phuong et al., 2023c). Thus, further research is necessary to determine the role of LvCTL3 in the immune response to pathogenic *Vibrio* spp. caused by AHPND.

To enhance lectin production for pathogenic control, several lectin-coding genes were cloned and heterozygous expression in hosts such as *E. coli*, *Pichia pastoris*, … In a study by Thao et al., (2020), a recombinant LvDLdrlCTL protein was able to agglutinate AHPND causing *V. parahaemolyticus.* Singrang et al., (2021) characterized a recombinant fibrinogen-related lectin PmFREP from the black tiger shrimp (*Penaeus monodon*) expressed in the insect (*Trichoplusia ni*) cell showed strong agglutinated *Pseudomonas aeruginosa* in a $Ca^{2+}$ ion-independent manner, but was not able to agglutinate with *V. parahaemolyticus*. However, the use of CTL in to enhance the innate immune response against infectious agents such as viruses or bacteria for animals including invertebrates is still further investigated (Zhang et al., 2017)

In conclusion, our experiments showed that the recombinant LvCTL3 from *L. vannamei* shrimp was expressed and functioned in *E. coli* BL21 StarTM (DE3) cells. The recombinant LvCTL3 protein exhibited strong agglutination with AHPND-causing *V. parahaemolyticus*. Therefore, the optimization of culture conditions for the recombinant LvCTL3 protein and its application in white-leg shrimp should be investigated.

## Declarations

**Declaration of Competing Interest**: The authors declare that they have no known competing financial interests or personal relationships that could have influenced the work reported in this paper

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