ISSN (O): 2581-8740 | ISSN (P): 2581-8732
Nguyen *et al*., 2020
DOI: 10.21276/SSR-IIJLS.2020.6.3.3

Research Article

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Cloning, Expression and Purification of *Vibrio parahaemolyticus* L-type Lectin from White Leg Shrimp *Litopenaeus vannamei* for Bacterial Agglutinating

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Received: 06 Jan 2020/ Revised: 02 Mar 2020/ Accepted: 10 Apr 2020

ABSTRACT

Background- Acute hepato pancreatic necrosis disease is the most severe disease currently affecting brackish-water shrimp aquaculture in Viet Nam, sourced by toxin-producing strains of *Vibrio parahaemolyticus*. In the Southwest of Vietnam, where more than 70% of Vietnamese shrimp production originated that the disease causes massive curses to shrimp farmers. Some previous published studies have proven that a new type of L-type lectins from *Litopenaeus vannamei,* named LvLTLC1, can support shrimps innate immune system to counteract the effects of *V. parahaemolyticus*.

Methods- In this study, a new L-type lectin (LvLTLC1)-encoding gene collected from *L. vannamei* was cloned into pET22b to generate recombinant pET-LvLTLC1 vector. Next, the vector was transformed into *E. coli* BL21 (DE3) expression host for protein expression.

Results- SDS-PAGE and Western blot immune probed with anti His-tag antibody showed that LvLTLC1 expressed in soluble form. With purity above 73%, recombinant LvLTLC1 protein generated from this study was used to assess the ability to agglutinate *V. parahaemolyticus* not depending on the attendance of calcium.

Conclusion- The results prompt us to evaluate not only the binding capacity, but also the facilitated bacterial clearance *in vivo* of the recombinant LvLTLC1 protein. The present work laid the ground work for pathogenic bacteria control in shrimp.

Key-words: AHPND, *Litopenaeus vannamei*, LvLTLC1, recombinant protein, *Vibrio parahaemolyticus*

INTRODUCTION

In 2012, there was massive loss in shrimp farming areas in the Southwest of Vietnam, of which the most dangerous is the acute hepatopancreatic necrosis disease (AHPND). According to the General Department of Fisheries (2013), the Southwest of Vietnam region accounts for 90.61% of brackish water shrimp farming area; 75.2% of production with 595,723 ha and 358,477 tons. However, with increasing intensity and increasing area, epidemics are becoming more and more widespread and difficult to control, seriously threatening productivity and sustainable development of shrimp farming [1]. The pathogen of AHPND in shrimp was identified as *V. parahaemolyticus* strain [2]. This strain of bacteria produces two toxins that rapidly destroy tissues and disrupt the hepatopancreas function in the shrimp's digestive system. These cause shrimp die prematurely within 30 days with mortality can reach over 70%. Shrimp can be infected with AHPND throughout the time of the culture, particularly up to 45 days old baby shrimp.

How to cite this article

Nguyen PTT, Nguyen LVH, Nguyen TT, Nguyen KLT, Tran-Van H. Cloning, Expression and Purification of *Vibrio parahaemolyticus* L-type Lectin from White Leg Shrimp *Litopenaeus vannamei* for Bacterial Agglutinating. SSR Inst. Int. J. Life Sci., 2020; 6(3): 2544-2551.

Access this article online

https://iijls.com/

ISSN (O): 2581-8740 | ISSN (P): 2581-8732
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DOI: 10.21276/SSR-IIJLS.2020.6.3.3

From the general epidemic situation mentioned above, the combination of many important factors to sustain development shrimp farming industry is necessary. These include research on improving the hatchery and shrimp production process, nutrition and environmental issues. At the same time, it is equally important to find out effective prevention and treatment measures.

Shrimp protecting themselves from serious infectious pathogens depend on the innate immune system like other invertebrates, consist of not only cellular react but also a humoral defense mechanism [3-5]. Encapsulation, nodule formation, and phagocytosis are involved the cellular [6]. Likewise, the connection between pattern recognition receptors (PRRs) and pathogen-associated molecular patterns (PAMPs) is an essential stage in the immune responses. Different PRRs recognize specific PAMPs and trigger signaling pathways of the immune responses. Surrounded by the diversity of PRRs, lectins have been identified as a potentially important agent in immune defense of shrimp [7]. C-type, M-type, L-type, P-type, fibrinogen-like domain lectins, calreticulin/ calnexin, and galectins are seven types of lectins were found in shrimp [6]. However, the insight of lectins function in the immune response system is still unclear, and most of the researches about shrimp lectins up to this point are concentrated on C-type lectin. Excepting LvLTLC1, a newest L-type lectin was identified from *L. vannamei*, exclusively MJLTL1 from tiger prawn *Marsupenaeus japonicus* [8] has been characterized [9].

L-type lectins, which contain a domain recognizing luminal carbohydrate can interact with N-glycans of glycoproteins [10]. A body of evidence has found and demonstrated the immunity support on cultured shrimp of LvLTLC1 when associated with some bacteria including *V. parahaemolyticus*, the pathogen of AHPND in shrimp, thus promising to be a potential source for AHPND treatment [9]. In Vietnam, there has been no research on the effective binding of *V. parahaemolyticus* of LvLTLC1 from white leg shrimp. In the present study, the recombinant L-type lectin from white leg shrimp *L. vannamei* was surveyed the ability of agglutination with AHPND-causing *V. parahaemolyticus*, which serves as the scientific premise for further studies on preventing and treating pathogenic bacteria diseases in shrimp.

MATERIALS AND METHODS

L. vannamei shrimps were selected from local farms in the Tan Phu Dong Town, Tien Giang Province, Vietnam and extracted at the Department of the Molecular and Environmental Biotechnology, Faculty of Biology and Biotechnology, University of Science, Nation University, Ho Chi Minh City, Vietnam from January 2018 to December 2019. This study isolated all the samples as stated by Tian *et al.* [9] with adoption.

RNA isolation and cDNA synthesis- White leg shrimps, weighing about 15 g, acquired from the previous day to prepare for the experiments. Grills were collected from healthy shrimp for RNA extraction [9]. RNA isolated by NucleoSpin RNA Plus kit (Macherey-Nagel, Germany) was used as the template for amplification of full-length cDNA. The cDNAs were synthesized using the MyTaq™ One-Step RT-PCR kit (Bioline, USA). Specific PCR primers (292F *Nde* and 293R *Xho*) shows in Table 1 and LvLTLC1 were designed for amplification of the encoding gene. One-step RT-PCR reaction was carried out as follows: 45 min at 45°C, 1 min at 95°C; and 30 cycles of 95°C for 15s, 55°C for 15s, 72°C for 10s; and extension for 10 min at 72°C. Then, aLvLTLC1 gene after purifying was cloned into a pET22b vector and sequenced by PhusaBiochem, Vietnam.

Table 1: Nucleotide sequences of primers used for amplification

Primers	Sequence(5'-3')
292F *Nde*	CATATGGATTACATGAAGCGAGAGCAC
293R *Xho*	CTCGAGGTGGAAATATCATATAATTTC
T7 promoter	TAATACGACTCACTATAGGG
T7 terminator	GCTAGTTATTGCTCAGCGG

***Underlined characters showed restriction enzyme**

Construction of pET22b-LvLTLC1 vector- LvLTLC1 gene was created using PCR method using MyTaq™ Red Mix (Bioline, USA), according to the recommend conditions: 95°C for 1 min, 30 cycles (95°C for 15s, 55°C for 15s and 72°C for 10s), and extension at 72°C for 10 min. LvLTLC1 was amplified using PCR with primer pairs (292FNde and 293RXho) are shown in Table 1. LvLTLC1 encoding gene was doubly digested with *NdeI and XhoI* (Thermo Scientific, USA) before being fused into respective restriction sites in the pET22b plasmid by using the T4 ligase (Thermo Scientific, USA). The ligation product was introduced into *E. coli* DH5α competent cells. Positive

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transformants were screened with T7 primers. The recombinant plasmid was named pET22b-LvLTLC1. Colony PCR analysis was done to identify the recombinant clones by using T7 primers on a vector.

Expression of LvLTLC1- The recombinant vector, pET22b-LvLTLC1, was introduced into the chemically competent *E. coli* BL21 (DE3) cells to express LvLTLC1 protein [11,12,16-20]. Bacteria cultured in LB-Amp (Luria-Bertani supplemented with 100 µg/ml ampicillin) medium until OD_{600} reached 0.4 to 0.6 before inducing without IPTG (in LB-Amp supplemented with 2% ethanol) for 20 h at 16^0C. Cells after harvested by centrifugation at 6,000 rpm for 5 min re-suspended in lysis buffer (0.5 M NaCl, 0.02 M NaH_2PO_4, 0.025 M imidazole, 2 mM dithiothreitol, 10% glycerol, and 1.5% Triton X-100) and disrupted by sonication (Microson Misonix incorporation, USA). The cell lysate was centrifuged at 13,000 rpm for 10 min at 4°C to collect the supernatant containing soluble proteins. Total proteins were analyzed by SDS-PAGE and Western blot immune probed with anti His-tag antibody.

LvLTLC1 purification- The supernatant after collected was put on 5 ml His-Trap column, which is combined with the FPLC (ÄKTA, GE Healthcare, USA) [9,16-20]. After washing step with binding buffer (0.5M NaCl, 0.02M NaH_2PO_4, 0.025M imidazole, 2 mM dithiothreitol, 10% glycerol, and 1.5% Triton X-100), eluted the recombinant proteins from His-Trap column by adding the elution buffer (binding buffer containing 0.5M imidazole). SDS-PAGE analyzed eluted protein fractions and the purity of the recombinant proteins was detected by the Gel-Pro Analyzer. The Bradford assay was used to determine protein concentration.

Evaluation the ability of agglutination with AHPND-causing *V. parahaemolyticus* of LvLTLC1- Gram-negative bacteria AHPND-causing *V. parahaemolyticus* were labeled at room temperature for 1 h with 2 µM Calcein AM, and re-suspended in TBS-Ca buffer (50 mM Tris–HCl, 100 mM NaCl, 10 mM $CaCl_2$, pH 7.5) at 10^6 CFU/ml. 10 µl of bacteria and 20 µl LvLTLC1 (0.1 mg/ml) or with a control GST protein (0.1 mg/ml) were blended together. Then incubating this mixture at 25°C for 1 h. Agglutination was observed under fluorescence microscope (Nikon, Japan) at 490 nm excitation [9]. To determine whether agglutination was calcium-independent, calcein AM-labeled microbe was incubated with LvLTLC1 in TBS buffer (TBS-Ca buffer without adding 10 mM $CaCl_2$) as described above.

RESULTS

RNA isolation and cDNA synthesis- Total RNA was successfully extracted from *L. vannamei* following the manufacturer's instructions (Fig. 1A, lane 1). This mRNA was used as the template for amplification of full-length cDNA. The cDNAs were synthesized using specific PCR primers (292F Nde and 293R Xho, Table 1) for LvLTLC1 gene. Using 1.5% agarose gel electrophoresis to separate the PCR product. The results of the analysis showed that we obtained only a band roughly at 678 bp fragment (Fig. 1B, lane 2), which was consistent with the size of the LvLTLC1 gene. In addition, the negative of the PCR reaction did not attend any contamination of PCR reaction (Fig. 1B, lane 1). This proved that the PCR reaction of the LvLTLC1 gene was not exogenous and the gene fragment was derived from the genome of *L. vannamei*. The DNA fragment was cloned into a pET22b vector and sequenced by PhusaBiochem, Vietnam.

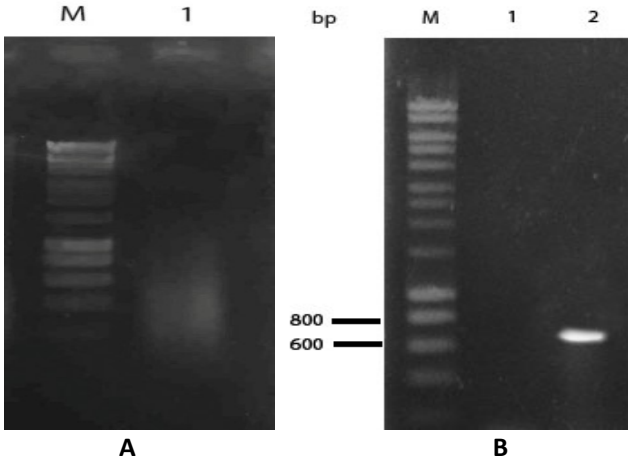

Fig. 1: RNA extraction and cDNA synthesis. RNA extraction
(A): Lane M, 1 kb DNA ladder; lane **1:** Shrimp RNAs. A fragment corresponding to the LvLTLC1 amplified by the RT-PCR
(B): Lane M, 1 kb DNA marker; lane **1:** Negative control; lane **2:** LvLTLC1 fragment

Construction of pET22b-LvLTLC1 vector- The construction of recombinant pET22b-LvLTLC1 was generated as described in materials and methods section. After the gene segments encoding LvLTLC1 were obtained through PCR, one band approximately at 678 bp was observed by agarose gel electrophoresis, indicated amplification of the DNA fragments isolated.

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The PCR fragment containing the LvLTLC1 gene was doubly digested with *NdeI* and *XhoI* restriction enzymes and then inserted into vector pET22b to obtain the expression plasmid pET22b-LvLTLC1. The ligated product was then transformed into *E. coli* strain (DH5α) and was selected on ampicillin-containing medium. By using the T7 primers on vector, colony PCR was chosen to confirm the recombinant colonies (Fig. 2). Colonies containing recombinant vector were given a 978-bp product. The results of electrophoresis showed that there were recombinant colonies suitable to the 978 bp length fragments as design (Fig. 2, lane 3-5). The positive result was due to the insertion of the LvLTLC1 gene into the plasmid pET22b.

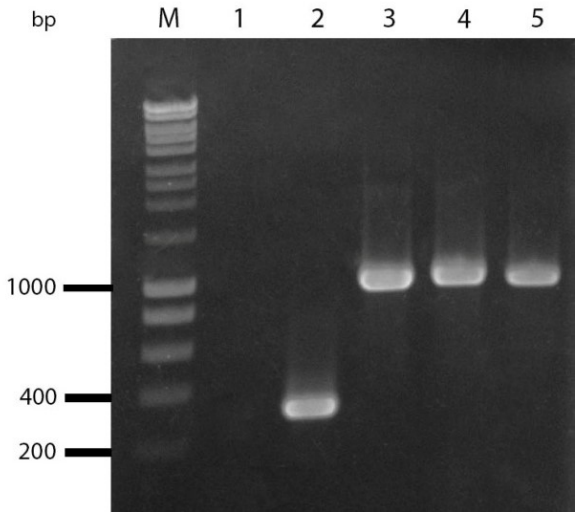

Fig. 2: Confirmation of recombinant clones of *E. coli* DH5α by colony PCR analysis with T7 primers. Lane M, 1 kb DNA ladder; lane 1, PCR negative control (no DNA); lane 2, negative control (*E. coli* DH5α/ pET22b); lane 3-5, candidate recombinant clones

To verify cloning precision, the vector was sequenced and aligned with the designed sequence. The alignment showed that the cloned genes were matched with the designed sequence and the LvLTLC1 gene was cloned (Fig. 3).

```
                1        11        21        31        41        51        61        71        81        91
LvLTLC1         ------GATTACATGAAGCGAGAGCACTCCCTCGTGAAGCCCTACCAAGGAATGGGTACTTCTATACCATACTGGGACTTCCTGGGTAACACTATGGTTA
clone 3         CATATGGATTACATGAAGCGAGAGCACTCCCTCGTGAAGCCCTACCAAGGAATGGGTACTTCTATACCATACTGGGACTTCCTGGGTAACACTATGGTTA
Consensus             gattacatgaagcgagagcactccctcgtgaagccctaccaaggaatgggtacttctataccatactgggacttcctgggtaacactatggtta

              101       111       121       131       141       151       161       171       181       191
LvLTLC1         CCAACAATTATATTCGTTTGACGGGAGATGTCCAGAGTGTGCGAGGAGCTGTTTGGAATAAGGTTCCGTGTTTCGTGCAAAATTGGGAGATGCAGATTCA
clone 3         CCAACAATTATATTCGTTTGACGGGAGATGTCCAGAGTGTGCGAGGAGCTGTTTGGAATAAGGTTCCGTGTTTCGTGCAAAATTGGGAGATGCAGATTCA
Consensus       ccaacaattatattcgtttgacgggagatgtccagagtgtgcgaggagctgtttggaataaggttccgtgtttcgtgcaaaattgggagatgcagattca

              201       211       221       231       241       251       261       271       281       291
LvLTLC1         GTTCAAAGTCCACGGTCGGGGGAAGGATTTGTTTGGTGACGGATTTGCCTTTTGGTATGTGAAAGATCCCATGGCTGAAGGTGATGTTTTCGGCAGCAAG
clone 3         GTTCAAAGTCCACGGTCGGGGGAAGGATTTGTTTGGTGACGGATTTGCCTTTTGGTATGTGAAAGATCCCATGGCTGAAGGTGATGTTTTCGGCAGCAAG
Consensus       gttcaaagtccacggtcgggggaaggatttgtttggtgacggatttgccttttggtatgtgaaagatcccatggctgaaggtgatgttttcggcagcaag

              301       311       321       331       341       351       361       371       381       391
LvLTLC1         GATTTCTTCACTGGATTGGGGGTTATTGCCGACACCTACAGCAACCACAATGGCCCACATAATCACGGTCACCCATACATTTCTGCGATGGTGAACAATG
clone 3         GATTTCTTCACTGGATTGGGGGTTATTGCCGACACCTACAGCAACCACAATGGCCCACATAATCACGGTCACCCATACATTTCTGCGATGGTGAACAATG
Consensus       gatttcttcactggattgggggttattgccgacacctacagcaaccacaatggcccacataatcacggtcacccatacatttctgcgatggtgaacaatg

              401       411       421       431       441       451       461       471       481       491
LvLTLC1         GTACTCTCCACTACGACCATGACCGAGACGGAACTCATACGCAGCTCTCTGGATGTGTGGCGAAGTTCAGGAATTTGGATTATGACACTTACCTGTCAAT
clone 3         GTACTCTCCACTACGACCATGACCGAGACGGAACTCATACGCAGCTCTCTGGATGTGTGGCGAAGTTCAGGAATTTGGATTATGACACTTACCTGTCAAT
Consensus       gtactctccactacgaccatgaccgagacggaactcatacgcagctctctggatgtgtggcgaagttcaggaatttggattatgacacttacctgtcaat

              501       511       521       531       541       551       561       571       581       591
LvLTLC1         CAGATACAAGCATGACACATTAACAGTTTCTATAGACATCGACAACAAGAGAGCGTTTAAGGAGTGCTTTACGGTGGGCGGAGTCATATTACCCACCGGA
clone 3         CAGATACAAGCATGACACATTAACAGTTTCTATAGACATCGACAACAAGAGAGCGTTTAAGGAGTGCTTTACGGTGGGCGGAGTCATATTACCCACCGGA
Consensus       cagatacaagcatgacacattaacagtttctatagacatcgacaacaagagagcgtttaaggagtgctttacggtgggcggagtcatattacccaccgga

              601       611       621       631       641       651       661       671       681       691
LvLTLC1         TACTACTTTGGCGTATCGGCTGCCACTGGGGATCTTAGCGATGCCCACGACCTAATATCAATGAAATTATATGATATTTCCACC------
clone 3         TACTACTTTGGCGTATCGGCTGCCACTGGGGATCTTAGCGATGCCCACGACCTAATATCAATGAAATTATATGATATTTCCACCCTCGAG
Consensus       tactactttggcgtatcggctgccactggggatcttagcgatgcccacgacctaatatcaatgaaattatatgatatttccacc
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Fig. 3: Sequence alignment between designed gene and a positive clone

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DOI: 10.21276/SSR-IIJLS.2020.6.3.3

Expression of LvLTLC1- The recombinant plasmid, pET22b-LvLTLC1was introduced into *E. coli* BL21 (DE3) competent cells which were sequenced using the T7 general primer on the vector, respectively (data not shown). An *E. coli* BL21 (DE3) colony containing pET22b-LvLTLC1 plasmid was induced protein expression without IPTG. Three samples were prepared from induced cells, including total protein samples, soluble samples, and insoluble sample. Negative control, *E. coli* BL21 (DE3), was induced and collected in the same order. All protein samples were analyzed by SDS-PAGE and Western blot. On the SDS-PAGE gel, the *E. coli* BL21 (DE3)/ pET22b-LvLTLC1 created an accumulated band (Fig. 4A, lane 2) less than 30 kDa, while negative control had no similar band (Fig. 4A, lane 1). This band was likely LvLTLC1protein. LvLTLC1 was not visibly detected in the insoluble lane (Fig. 4A, lane 4).

To assure the protein bands on the SDS-PAGE were LvLTLC1, a Western blot with anti 6xHis antibody was performed. Because the LvLTLC1 recombinant protein was designed with 6xHis at the C-terminal; therefore, it gives a signal on western blot. In the western-blot film, the total and the soluble samples had one band for each (Fig. 4B, lane 2-3), with sizes correlated to bands on the SDS-PAGE gel, and suggested that the proteins were successfully blotted into the membrane. In conclusion, the protein expressed by *E. coli* BL21 (DE3)/ pET22b-LvLTLC1 was LvLTLC1 protein.

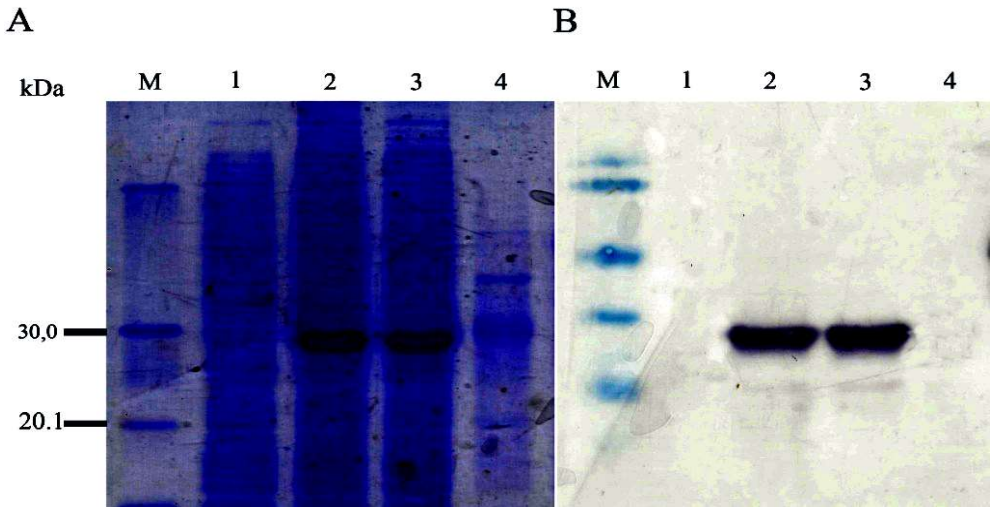

Fig. 4: Analysis of LvLTLC1; **(A):** SDS-PAGE, **(B):** Western blot
Lane M, ladder; lane **1:** Negative control; **2:** Total protein samples; **3:** Soluble samples; **4:** Insoluble samples

LvLTLC1 purification- After purification, four fractions, including expressed protein sample, flow-through of loading-step, washing-step, and elution-step samples were evaluated by SDS-PAGE. The results (Fig. 5) indicated that the LvLTLC1protein was successfully purified. There was only an accumulated band in the elution sample less than 30 kDa (Fig. 5, lane 4-6) correlated with an over expressed band of the original sample (Fig. 4, lane3). The purity analysis results were performed using Gel Analyzer software. As such, we have initially purified and successfully obtained recombinant LvLTLC1 protein with 73.96%.

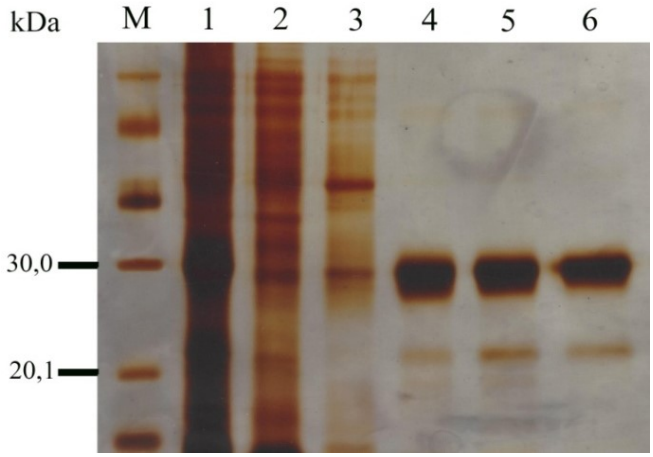

Fig. 5: Purification of LvLTLC1 analyzed by SDS-PAGE. Lane M, ladder; lane **1:** Total protein samples; Lane **2:** Flow-through sample; Lane **3:** Washing sample; Lane **4-6:** Elution samples

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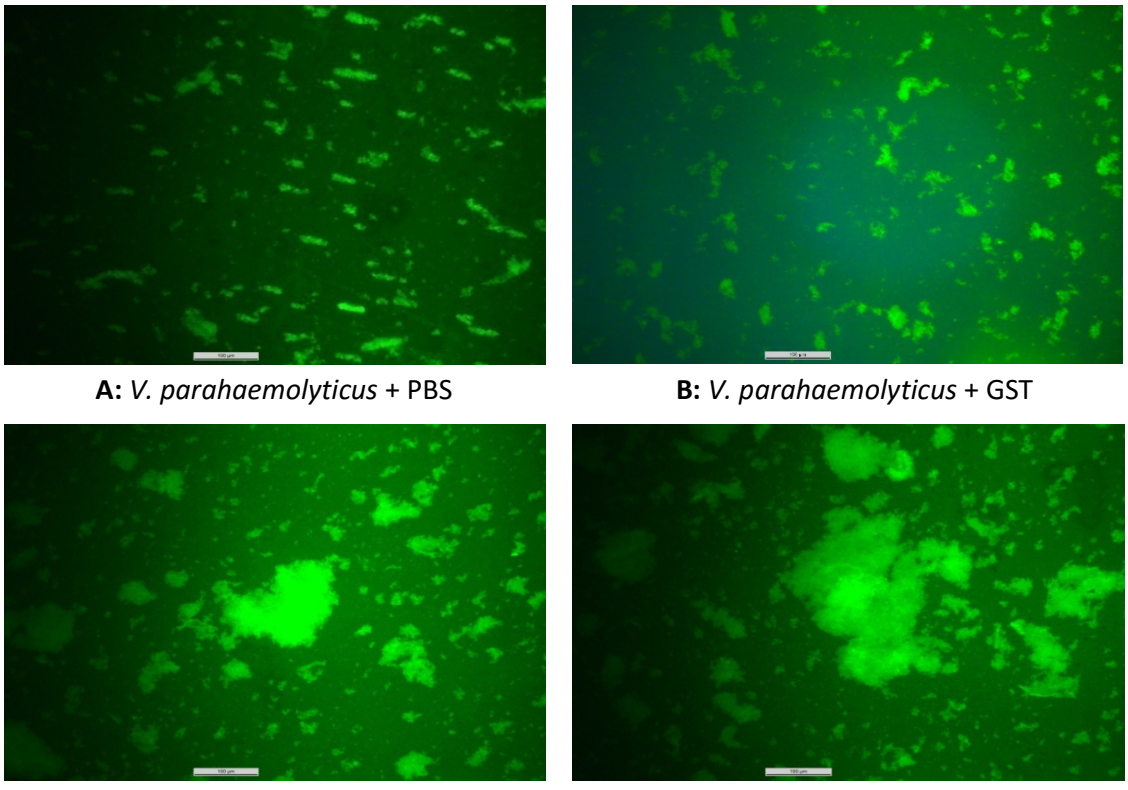
Evaluation the ability of agglutination with AHPND-causing *V. parahaemolyticus* of LvLTLC1- After incubation with the recombinant protein, bacterial agglutination reaction was observed under a fluorescence microscope. Research results have shown that *V. parahaemolyticus* could be agglutinated by LvLTLC1 (Fig. 6C, 6D), and could not be agglutinated by control protein GST (Fig. 6B). The agglutination capabilities of LvLTLC1 could be clearly observed not depending on the presence of calcium (Fig. 6C) suggested that LvLTLC1 could agglutinate in calcium-independent manner. However, enhanced agglutinating activity shown in bigger agglutinating clumps (Fig. 6D compares to Fig. 6C), when calcium was added.

Evaluation the agglutination ability of LvLTLC1 with AHPND-causing *V. parahaemolyticus*- After incubation with the recombinant protein, bacterial agglutination was observed under fluorescent microscope. The results showed that *V. parahaemolyticus* could be agglutinated by LvLTLC1 (Fig. 6C, 6D), and could not be agglutinated by control protein GST (Fig. 6B). The agglutinating activity of LvLTLC1 could be observed without the presence of calcium (Fig. 6C) suggested that LvLTLC1 could agglutinate in calcium-independent manner. However, the addition of calcium enhanced agglutinating activity shown in bigger agglutinating clumps (Fig. 6D compares to Fig. 6C).



A: *V. parahaemolyticus* + PBS

B: *V. parahaemolyticus* + GST

C: *V. parahaemolyticus* + LvLTLC1

D: *V. parahaemolyticus* + LvLTLC1+Ca^{2+}

Fig. 6: *V. parahaemolyticus* agglutinated by LvLTLC1

DISCUSSION

In this study, the several experimental tests have shown that LvLTLC1 was non-classical inclusion bodies. From these results, sequential steps for increasing the solubility of protein LvLTLC1 were considered. The solubility of protein could be enhanced, when reducing the rate of protein synthesis and reducing the number of hydrophilic interactions may interfere with proper folding of the protein. Firstly, the culture medium supplemented with ethanol [11,12] caused cell to grow slowly down because ethanol had the same effect as the stress agent on *E. coli* [13]. Attaching to some small chemical agents could make expression proteins to be probable and widespread application have found in recombinant protein productions [14]. This hypothesis was exploited to increase the proportion of recombinant protein expressed in the soluble form [15]. Secondly, the expression was led overnight at lower temperature (as low as16^{o}C) with no IPTG added to expressed LvLTLC1

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because leaky promoter in pET vector causing the gradual secretion of protein into cytoplasm. Thirdly, 10% glycerol, 1.5% Triton X-100, and 2 mM dithiothreitol were supplemented with sonication buffer [16]. Because of glycerol together with a slow shake could help reduce the number of hydrophilic interactions that may interfere with the proper folding of protein [17,18], and Triton X-100 is a well-known surfactant that increases the solubility of the hydrophobic compound [19]. Finally, reducing agent with low concentration when added such as DTT not only prevent the formation of wrong disulfide bonds, but also keeps cysteine side chains in the reduced state. This role analysis buffer by maintaining side chain and protecting the creation of unpredicted disulfide bonds [20].

Before LvLTLC1, like almost of C-type lectins which are classical calcium-dependent lectins [21,22], L-type lectins have a domain which is binding to calcium [23]. In the results presented here, LvLTLC1 promoted the capacity of agglutination with V. *parahaemolyticus* not depending on the attendance of calcium, and the activities of LvLTLC1 were increased when calcium presented. An interesting phenomenon was that LvLTLC1 contains not only maintained calcium-bonding residues of L-type lectin [24] but some differences in amino acid residues, which probably determined the calcium-independence of LvLTLC1 [8]. This hypothesis is in line with L-type calcium-dependent lectin MJLTL1 [8] although the calcium-independent lectins-binding carbohydrates via other mechanisms are not fully understood.

CONCLUSIONS

This research successfully structured the recombinant vector carrying the LvLTLC1 gene (pET-LvLTLC1), which codes LvLTLC1 protein derived from *L. vannamei*; successfully formed *E. coli* BL21 (DE3) strain carrying pET-LvLTLC1 vector capable of expressing recombinant LvLTLC1 protein with 73.96% purity. Besides, we tested the ability to bind *V. parahaemolyticus* pathogenic bacteria of LvLTLC1. Data showed that LvLTLC1 could agglutinate AHPND-causing *V. parahaemolyticus* not depending on the attendance of calcium.

On the other hand, the product from this research could be used as a supply of recombinant protein LvLTLC1 for further researches in evaluating the immune-supporting activity of LvLTLC1, towards the development of products preventing and treating AHPND in shrimp.

ACKNOWLEDGMENTS

This research was supported by a grant funded by the Tien Giang University (Grant number: T2015-14).

CONTRIBUTION OF AUTHORS

Research concept- Phuong-Thao Thi Nguyen, Hieu Tran-Van

Research design- Phuong-Thao Thi Nguyen, Hieu Tran-Van

Supervision- Hieu Tran-Van

Materials- Kim-Loan Thi Le, Phuong-Thao Thi Nguyen, Hieu Tran-Van

Data collection- Phuong-Thao Thi Nguyen, Lan-Vy Hoai Nguyen, Thang Tan Nguyen, Kim-Loan Thi Le

Data analysis and Interpretation- Phuong-Thao Thi Nguyen, Lan-VyHoai Nguyen, Thang Tan Nguyen, Kim-Loan Thi Le, Hieu Tran-Van

Literature search-Phuong-Thao Thi Nguyen, Lan-Vy Hoai Nguyen

Writing article, critical review, article editing-Phuong-Thao Thi Nguyen, Hieu Tran-Van

Final approval- Hieu Tran-Van

REFERENCES

[1] Flegel TW. Historic emergence, impact and current status of shrimp pathogens in Asia. J. Invertebr. Pathol., 2012; 110(2): 166-73.

[2] Tran L, Nunan L, Redman RM, Mohney LL, Pantoja CR, et al. Determination of the infectious nature of the agent of acute hepatopancreatic necrosis syndrome affecting penaeid shrimp. Dis. Aquat. Organ., 2013; 105(1): 45-55.

[3] Li F, Xiang J. Recent advances in researches on the innate immunity of shrimp in China. Dev. Comp. Immunol., 2013; 39(1-2): 11-26.

[4] Walker PJ, Winton JR. Emerging viral diseases of fish and shrimp. Vet. Res., 2010; 41(6): 51.

[5] De Schryver P, Defoirdt T, Sorgeloos P. Early Mortality Syndrome Outbreaks: A Microbial Management Issue in Shrimp Farming?. PLoS. Pathog., 2014;10(4): e1003919.

[6] Iwanaga S, Lee BL. Recent advances in the innate immunity of invertebrate animals. J. Biochem. Mol. Biol., 2015; 38(2): 128-50.

[7] Nyholm SV, Graf J. Knowing your friends: Invertebrate innate immunity fosters beneficial bacterial symbioses. Nat. Rev. Microbiol., 2012; 10(12): 815.

ISSN (O): 2581-8740 | ISSN (P): 2581-8732
Nguyen *et al*., 2020
DOI: 10.21276/SSR-IIJLS.2020.6.3.3

[8] Xu S, Wang L, Wang XW, Zhao YR, Bi WJ, et al. L-Type lectin from the kuruma shrimp *Marsupenaeus japonicus* promotes hemocyte phagocytosis. Dev. Comp. Immunol., 2014; 44(2): 397-405.

[9] Tian Y, Chen T, Huang W, Luo P, Huo D, et al. A new L-type lectin (LvLTLC1) from the shrimp *Litopenaeus vannamei* facilitates the clearance of *Vibrio harveyi*. Fish. Shellfish. Immunol., 2018;73: 185-91.

[10]Kamiya Y, Kamiya D, Yamamoto K, Nyfeler B, Hauri HP, et al. Molecular basis of sugar recognition by the human L-type lectins ERGIC-53, VIPL, and VIP36. J. Biol. Chem., 2008; 283(4): 1857-61.

[11]Dinnbier U, Limpinsel E, Schmid R, Bakker EP. Transient accumulation of potassium glutamate and its replacement by trehalose during adaptation of growing cells of *Escherichia coli* K-12 to elevated sodium chloride concentrations. Arch. Microbiol., 1988; 150(4): 348-57.

[12]Nieba L, Honegger A, Krebber C, Plückthun A. Disrupting the hydrophobic patches at the antibody variable/constant domain interface: Improved in vivo folding and physical characterization of an engineered scFv fragment. Protein. Eng., 1997; 10(4): 435-44.

[13]Chhetri G, Kalita P, Tripathi T. An efficient protocol to enhance recombinant protein expression using ethanol in *Escherichia coli*. Methods X., 2015; 2: 385-91.

[14]Graslund S, Nordlund P, Weigelt J, Hallberg BM, Bray J, et al. Protein production and purification. Nat. Methods, 2008; 5(2): 135.

[15]Grunberg-Manago M. Messenger RNA Stability and Its Role in Control of Gene Expression in Bacteria and Phages. Annu. Rev. Genet., 1999; 33(1): 193-227.

[16]Esmaili I, Mohammad Sadeghi HM, Akbari V. Effect of buffer additives on solubilization and refolding of reteplase inclusion bodies. Res. Pharm. Sci., 2018; 13(5): 413.

[17]Vagenende V, Yap MG, Trout BL. Mechanisms of protein stabilization and prevention of protein aggregation by glycerol. Biochem., 2009;48(46): 11084-96.

[18]Al-Samarrai TH, Jones WT, Harvey D, Kirk CA, Templtone M. Effect of 4% glycerol and low aeration on result of expression in *Escherichia coli* of Cin3 and three Venturia EST's recombinant proteins. Am. J. Mol. Biol., 2013; 3: 1-9.

[19]Farsang E, Gaal V, Horvath O, Bardos E, Horvath K. Analysis of non-ionic surfactant Triton X-100 using hydrophilic interaction liquid chromatography and mass spectrometry. Mol., 2019; 24(7): 1223.

[20]Kelly ST, Zydney AL. Effects of intermolecular thiol-disulfide interchange reactions on bsa fouling during microfiltration. Biotechnol. Bioeng., 1994; 44(8): 972-82.

[21]Cambi A, Koopman M, Figdor CG. How C-type lectins detect pathogens. Cell. Microbiol., 2005; 7(4): 481-88.

[22]Zelensky AN, Gready JE. The C-type lectin-like domain superfamily. FEBS. J., 2005; 272(24): 6179-17.

[23]Hart GW, Akimoto Y. Essentials of Glycobiology. 2nd Ed., USA; Cold Spring Harbor Laboratory Press, 2009.

[24]Mojica ER, Merca FE. Lectin from the Body Walls of Black Sea Cucumber (*Holothuria atra* Jaeger). Philipp. J. Sci., 2004; 133(2): 77.