

Article

Expression of lysozyme in diseased shrimp collected from two different coastal areas

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Abstract: Lysozyme (*lyso*) is an anti-microbial peptide (AMP) found in Eukaryotes. In shrimps, lysozyme gene is expressed for protection against microbial infection. This current study targeted to determine the differences in expression in the samples from two different coastal regions having spatial variation for comparison. In the current study, diseased shrimps were collected from improved traditional ghers of Satkhira and Cox's Bazar districts which are two different coastal areas of Bangladesh. Healthy shrimps were also collected from the ghers of those regions. Average relative expression of *lyso* gene in diseased shrimps from Satkhira region was lower than that of Cox's Bazar region. Average fold-differences were observed 1.9, 2.3, and 2.5 in the shrimps collected from the ghers of Satkhira, and it was observed 2.6, 2.9 and 2.7 in the shrimp ghers of Cox's Bazar. This study supports the hypothesis that immune-related gene expression might vary in different farms and regions in variations of water, environment and biological parameters for the state of the host.

Keywords: diseased shrimp; lysozyme; gene expression; coastal areas

1. Introduction

In the economic development of a number of countries including Bangladesh, shrimp aquaculture performs a very important part (Shabuj *et al.*, 2016). The inhabitants of coastal zone of several nations solely depend on shrimp and crab culture. When shrimps are impacted due to diseases, individuals reliant on aquaculture have to look for other income generating activities which head towards socioeconomic concerns affecting the environmentally sustainable shrimp aquaculture (Paul and Vogl, 2012). The economic cost of a particular single disease, known as White Spot Disease on the shrimp farming industry was assessed up to US\$15 billion as it initially arose expanding at the rate of US\$1 billion yearly (Stentiford *et al.*, 2012). Nevertheless, shrimp farming had developed to be the utmost profitable division in aquaculture businesses worldwide (Hasan *et al.*, 2022). Numerous causative agents are responsible for various shrimp diseases including white spot syndrome virus (WSSV), yellow head virus (YHV), Taura syndrome virus (TSV), and bacteria from the genus *Vibrio*, which create massive loss in the shrimp aquaculture industry (Lightner, 2011; Tassanakajon *et al.*, 2013). Infection is continually a crisis which annoys the healthful growth of shrimp culture. Virus and bacteria both can be equally serious disease-causing agents in shrimp culture farms, especially in the improved traditional ones. Use of conventional antibiotics is capable of alleviating bacterial disease, but outdated strategies applied to

avoid diseases caused by viruses in vertebrates are not efficient to remedy viral diseases of shrimps as there is no adaptive immunity in such crustaceans (Li and Xiang, 2013).

Before 2000, there had not been many studies on the innate immunity of shrimps. Clear insight on the crustacean immunology is needed for developing an active policy to control disease. The explicit or implicit identification of pathogenic organisms or pathogen-associated molecular patterns (PAMPs) by pattern recognition receptors directs towards quick humoral and cellular resistance reactions (Li and Xiang, 2013). Resistance of shrimps through their innate immunity were lately explored by researchers (Li and Xiang, 2013; Tassanakajon *et al.*, 2013). In aquatic organisms like fish and shrimp, the innate immune response system is the primary shield that opposes morbidic infections (Magnadóttir, 2006). Numerous factors and genes were observed as immunological pointers. The shifts in leukocyte amounts and total concentrations of serum protein, albumin, and globulin were extensively employed for the demonstration of tissue injury and diagnosing disorders in aquatic organisms (Elasely *et al.*, 2014; Yang *et al.*, 2015).

Lysozyme, which is an N-acetylmuramide glycanohydrolase or muramidase, exists as a bacteriolytic catalyst working like an antibody in the eukaryote (Magnadóttir, 2006). Lysozyme from the parent has been observed to help fish that lack matured immune system due to its role against the infection of the *Aeromonas salmonicida* (Magnadóttir *et al.*, 2005). Wang *et al.* (2018) found changes in the activity of lysozyme in the in-vivo assays with different salinity stressed conditions. Hasan *et al.* (2022) mentioned lysozyme as a very critical AMP participating in the host-pathogen interactions which may change expression patterns when infected with virus. Therefore, the current study targeted to observe the relative expression of lysozyme in diseased penaeid shrimps collected from Satkhira and Cox's Bazar which are different geographical regions.

2. Materials and Methods

2.1. Sample collection and measurement of the physico-chemical parameters

The shrimp species collected for the current study were *Penaeus monodon*, the black tiger shrimp farmed in the coastal areas of Bangladesh. Diseased shrimps were collected from three improved traditional gher of Kaliganj Upazilla of Satkhira district (K1, K2, K3) and three improved traditional gher of Cox's Bazar Sadar Upazilla of Cox's Bazar district (CS1, CS2, CS3). Fresh shrimp samples were collected from the gher (K4, K5, K6 and CS4, CS5, CS6) where no disease was reported in the last two years. Physico-chemical parameters, temperature, salinity, dissolved oxygen and pH were measured from the surface water of three different points of the gher.

2.2. Preparation of samples, RNA Extraction and cDNA synthesis

Samples after collection were immediately brought to the laboratory for extracting RNA. For the purpose of RNA extraction, tissue samples were collected from the below part of cephalothorax region. RNA extraction kit (New England Biolabs, Ipswich, MA, USA) was utilized for extracting RNA from diseased and fresh shrimps. PhotoScript II First Strand cDNA Synthesis Kit, (New England Biolabs, Ipswich, MA, USA) was used for the synthesis of cDNA (Hasan *et al.*, 2022).

2.3. Gene expression analysis

Relative expressions of lysozyme gene were observed using real-time PCR. Real-time PCR conditions followed the methods described by Hasan *et al.*, (2022). House-keeping gene (internal control), β -actin, was used for the normalization purpose. Fresh shrimp samples were used as control (exogenous control). Table 1 enlisted the primers utilized for the gene expression analysis experiments using the real-time PCR where samples were run in triplicate. For the analysis of average-fold difference in gene expression, comparative delta C_T method was used (Jeena *et al.*, 2012). Fold-differences in gene expression were statistically confirmed at 5% level of significance smearing t-test.

Table 1. Primers that were used in the study.

Gene	Primer sequence (5'-3')	Reference
β -actin	F: CCCTGTTCCAGCCCTCATT R: GGATGTCCACGTCGCACTT	Shekhar <i>et al.</i> , 2015
Lysozyme	F: TGGTGTGGCAGCGATTATG R: GATCGAGGTCGCGATTCTTAC	Deris <i>et al.</i> , 2020

3. Results and Discussion

Average fold difference of relative expression of lysozyme was 1.9, 2.3, and 2.5 in K1, K2, and K3 gher of Kaliganj, Satkhira, respectively. On the other hand, average fold difference was 2.6, 2.9 and 2.7 in CS1, CS2 and CS3 gher of Cox's Bazar Sadar, Cox's Bazar, respectively (Figure 1). There was a significant difference ($p < 0.05$) in the average relative expression of lysozyme between the control (healthy samples collected from K4, K5, K6, CS4, CS5, CS6) and the diseased shrimps from both areas. However, the expression was found to be lower in the samples of Satkhira than Cox's Bazar. Physico-chemical parameters were found to vary with a considerable range showed in Table 2. Temperature, salinity, pH and DO are key variables in coastal water. The average highest temperature was observed in CS4 and the average lowest temperature was observed in K1 and K5. Lowest salinity was observed in K2 while the highest was in CS6. In the CS6, the amount of dissolved oxygen was found to be highest while the lowest was observed in K1. The highest and lowest average pH were observed in CS6 and CS2, respectively.

Table 2. Mean values of the physico-chemical parameters of the improved traditional gher of the sampling areas.

Gher ID	Temperature	Salinity	Dissolved oxygen	pH
K1	30.5±1.33	14±1	3.9±0.4	7.8±0.2
K2	31.7±1.67	12±1.5	4.2±0.45	8±0.1
K3	30.8±1.23	12.9±1.1	4.5±0.33	8.4±0.3
K4	31±2.1	15±1.5	5.3±0.61	8.3±0.1
K5	30.5±1.77	15.5±1	4.9±0.43	8.1±0.3
K6	30.9±1.45	16±1.5	5±0.32	7.9±0.15
CS1	32±1	14.7±1.3	4.8±0.5	8±0.4
CS2	31.6±1.14	14.9±2.4	4.9±0.41	7.6±0.05
CS3	31.8±1.66	14±2	5.5±0.52	7.7±0.14
CS4	32.2±1.43	14.9±1.9	5.1±0.44	8.2±0.22
CS5	31.4±1.56	15.5±1.5	4.5±0.5	8.5±0.42
CS6	31.7±2	17±1	6±0.66	8.6±0.2

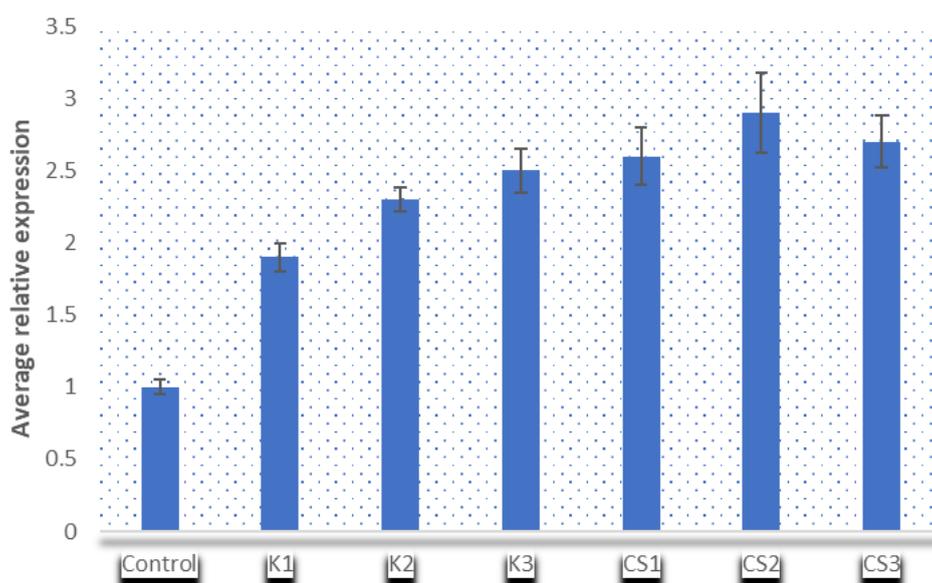


Figure 1. Average relative expression of lysozyme gene in the diseased penaeid shrimps collected from improved traditional gher of Satkhira (K1, K2, K3) and Cox's Bazar (CS1, CS2, CS3) normalized with β -actin as house-keeping gene and exogenous control (healthy shrimps collected from K4, K5, K6 and CS4, CS5, CS6).

Abiotic factors were estimated to be affecting gene expressions. The lysozyme expression was impacted by increase or decrease in abiotic factors (Wang *et al.*, 2018). There are effects of physico-chemical parameters on

the immunity of shrimps. Shrimps seem to behave similarly for the immune response like insects and it was found that the IMD (immune deficiency) pathway silencing resulted in dramatical decrease in lysozyme expression level. Shrimp is frequently afflicted with *Vibrio*, and it is established that the shrimp lysozyme like chicken lysozyme (c-lyz) works for the protection against the bacteria of this genus (Peregrino-Uriarte *et al.*, 2012). Lysozyme is treated as an additive which works against pathogenic microorganisms in dairy foods (Cosentino *et al.*, 2016). Lysozyme release slowed down at pH 7 due to the exchanges between cationic muramidase and non-ionic API or Na-caseinate (Benelhadj *et al.*, 2016).

Hasan *et al.* (2022) tested lysozyme in experimentally infected shrimps with different doses and with different phylogroups of the virus and observed variations in average relative expression between the infected shrimps with two groups of virus. Deris *et al.* (2020) observed different patterns of lysozyme gene expressions in shrimp post-larvae (PL) after challenged with pathogenic microorganisms. Shrimps infected with *Vibrio parahaemolyticus* and *V. harveyi* supported through higher expression of lysozyme and other genes that PL of later stages could be more vulnerable to infections (Deris *et al.*, 2020; Utiswannakul *et al.*, 2011). Although no significant association was observed, there was variability found in the physico-chemical parameters of gher from which healthy shrimps were collected and from those in which diseased shrimps were found in the current study. Moreover, size, age and maturity could be among different other factors for the variation in gene expression (Vinuela *et al.*, 2018).

4. Conclusions

In this study, lysozyme expression was observed in the diseased shrimps which were collected after farmers' report of the presence of diseased shrimps in their improved traditional gher. Two geographically different regions of the country were selected where shrimps are cultured since long. The expression of this anti-microbial peptide differed significantly. The reasons behind this variation could be due to the several physico-chemical parameters of the gher which might vary between regions and also for the physiological state in which the shrimps were collected for the analysis of gene expression.

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Data availability

Ct values for the calculation of gene expression analysis is available on request.

Conflict of interest

None to declare

Authors' contributions

Mehedi Mahmudul Hasan primarily planned the work. After the initial planning, Mehedi Mahmudul Hasan and Md. Anisuzzaman conducted the study. All authors have read and approved the final manuscript.

References

- Benelhadj S, N Fejji, P Degraeve, H Attia, D Ghorbel and A Gharsallaoui, 2016. Properties of lysozyme/*Arthrospira platensis* (Spirulina) protein complexes for antimicrobial edible food packaging. *Algal Res.*, 15:43-49.
- Cosentino C, HS Elshafie, I Camele, M Musto, R Paolino, C D'Adamo and P Freschi, 2016. Effects of different heat treatments on lysozyme quantity and antimicrobial activity of jenny milk. *Journal of Dairy Science*, 99: 5173-5179.
- Deris ZM, S Iehata, M Ikhwanuddina, MBMK Sahimi, TD Do, P Sorgeloos, YY Sung and LL Wong, 2020. Immune and bacterial toxin genes expression in different giant tiger prawn, *penaeus monodon* post-larvae stages following AHPND-causing strain of *vibrio parahaemolyticus* challenge. *Aquac. Rep.*, 16: 100248.
- Elasely AM, AA Abbass and B Austin, 2014. Honey bee pollen improves growth, immunity and protection of Nile tilapia (*Oreochromis niloticus*) against infection with *Aeromonas hydrophila*. *Fish Shellfish Immunol.*, 40: 500-506.
- Hasan MM, MN Hoque, F Ahmed, MI-M Haque, M Sultana and MA Hossain, 2022. Circulating Phylotypes of White Spot Syndrome Virus in Bangladesh and Their Virulence. *Microorganisms*, 10: 191.

- Jeena K, KP Prasad, MK Pathan and PG Babu, 2012. Expression Profiling of WSSV ORF 199 and Shrimp Ubiquitin Conjugating Enzyme in WSSV Infected *Penaeus monodon*. Asian-Australas J. Anim. Sci., 25: 1184 – 1189.
- Li F and J Xiang, 2013. Recent advances in researches on the innate immunity of shrimp in China. Develop. Compar. Immunol., 39: 11-26.
- Magnadottir B, S Lange, S Gudmundsdottir, J Bøggwald and RA Dalmo, 2005. Ontogeny of humoral immune parameters in fish. Fish Shellfish Immunol., 19: 429–439.
- Magnadóttir B. Innate immunity of fish (overview). Fish Shellfish Immunol., 20: 137–151.
- Paul BG and CR Vogl, 2012. Key performance characteristics of organic shrimp aquaculture in Southwest Bangladesh. Sustainability, 4: 995–1012.
- Peregrino-Uriarte AB, AT Muhlia-Almazan, AA Arvizu-Flores, G Gomez-Anduro, T Gollas-Galvan, G Yepiz-Plascencia and RR Sotelo-Mundo, 2012. Shrimp invertebrate lysozyme i-lyz: gene structure, molecular model and response of c and i lysozymes to lipopolysaccharide (LPS). Fish Shellfish Immunol., 32: 230-236.
- Shabuj MAI, T Bairagi, A Al-Asif, O Faruq, MR Bari and MS Neowajh, (2016). Shrimp disease investigation and culture strategies in Bagerhat district, Bangladesh. Asian J. Med. Biol. Res., 1: 545–552.
- Shekhar MS, A Gomathi, G Gopikrishna and AG Ponniah, 2015. Gene expression profiling in gill tissues of White spot syndrome virus infected black tiger shrimp *Penaeus monodon* by DNA microarray. Virus Dis., 26: 9–18.
- Stentiford G, D Neil, E Peeler, J Shields, H Small, T Flegel, J Vlcek, B Jones, F Morado, S Moss, J Lotz, L Bartholomay, D Behringer, C Hauton and D Lightner, 2012. Disease will limit future food supply from global crustacean fishery and aquaculture sectors. J. Inver. Pathol., 110: 141-157.
- Tassanakajon A, K Somboonwiwat, P Supungul and S Tang, 2013. Discovery of immune molecules and their crucial functions in shrimp immunity. Fish Shellfish Immunol., 34: 954-967.
- Utiswannahkul P, S Sangchai and S Rengpipat, 2011. Enhanced growth of black tiger shrimp *Penaeus monodon* by dietary supplementation with *Bacillus* (BP11) as a probiotics. J. Aquac. Res. Development, S1: 006.
- Viñuela A, AA Brown, A Buil, PC Tsai, MN Davies, JT Bell, ET Dermitzakis, TD Spector and KS Small, 2018. Age-dependent changes in mean and variance of gene expression across tissues in a twin cohort. Hum. Mol. Genet., 27: 732-741.
- Wang D, Q Cao, W Zhu, Y Hu, X Zhang, S Yin and T Wang, 2019. Individual and combined effects of salinity and lipopolysaccharides on the immune response of juvenile *Takifugu fasciatus*. Fish Physiol. Biochem., 45: 965–976.
- Yang X, JL Guo, JY Ye, YX Zhang and W Wang, 2015. The effects of *Ficus carica* polysaccharide on immune response and expression of some immune-related genes in grass carp, *Ctenopharyngodon idella*. Fish Shellfish Immunol.: 42: 132–137.