



Anti-lipopolysaccharide factor and crustin-III, the anti-white spot virus peptides in *Penaeus monodon*: Control of viral infection by up-regulation

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ABSTRACT

White Spot Syndrome Virus (WSSV) is the most devastating disease affecting shrimp culture around the world. Though, considerable progress has been made in the detection and molecular characterization of WSSV in recent years, information pertaining to immune gene expression in shrimps with respect to WSSV infection remains limited. In this context, the present study was undertaken to understand the differential expression of antimicrobial peptide (AMP) genes in the haemocytes of *Penaeus monodon* in response to WSSV infection on a time-course basis employing semi-quantitative RT-PCR. The present work analyzes the expression profile of six AMP genes (ALF, crustin-1, crustin-2, crustin-3, penaeidin-3 and penaeidin-5), eight WSSV genes (DNA polymerase, endonuclease, immediate early gene, latency related gene, protein kinase, ribonucleotide reductase, thymidine kinase and VP28) and three control genes (18S rRNA, β -actin and ELF) in *P. monodon* in response to WSSV challenge. Penaeidins were found to be up-regulated during early hours of infection and crustin-3 during late period of infection. However, ALF was found to be up-regulated early to late period of WSSV infection. The present study suggests that AMPs viz. ALF and crustin-3 play an important role in antiviral defense in shrimps. WSSV gene transcripts were detected post-challenge day 1 itself and increased considerably day 5 onwards. Evaluation of the control genes confirmed ELF as the most reliable control gene followed by 18S rRNA and β -actin for gene expression studies in shrimps. This study indicated the role of AMPs in the protection of shrimps against viral infection and their possible control through the up-regulation of AMPs.

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1. Introduction

White spot disease (WSD) caused by white spot syndrome virus (WSSV) is currently the most serious viral pathogen of shrimps worldwide causing 100% mortality within 7–10 days of infection (Sanchez-Paz, 2010). Although considerable progress has been made in the characterization of WSSV, little work has been done on the host immune response particularly by haemocytes in response to the viral infection at the molecular level (Soderhall and Cerenius, 1992; Wongpanya et al., 2007). Antimicrobial peptides (AMPs) which constitute innate immune defense mechanism, have been a subject of intense research in the last few decades; regarding their biosynthesis, activity against microorganisms, mechanism of action and potential clinical applications. In penaeid shrimps, three main families of AMPs have been currently described and characterized from the haemocytes: anti lipopolysaccharide factors (ALF), crustins

and penaeidins (Rosa and Barracco, 2010; Tincu and Taylor, 2004). The present study was undertaken to analyze the differential expression of AMP genes and WSSV genes in the haemocytes of *Penaeus monodon* in response to WSSV infection on a time-course basis employing semi-quantitative RT-PCR. We hope the present study might generate new premise for understanding shrimp defense against WSSV infection in terms of AMP gene expression. To our knowledge this is the first report on the time-course analysis of AMP gene expression in the haemocytes of *P. monodon* in response to WSSV challenge.

2. Materials and methods

2.1. Experimental animals and rearing conditions

Healthy adult *P. monodon* (45–50 g body weight) collected from a local shrimp farm in Vypeen, Kochi were used for the study. The animals were kept in aquarium tanks of 500 l capacity and acclimatized for one month under laboratory conditions. Shrimps were fed standard diet (Higashi, India) twice daily ad libitum. Physico-chemical parameters such as salinity, pH, alkalinity, ammonia, nitrite, nitrate, dissolved oxygen and temperature were monitored daily and

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maintained at optimal levels (Table 1). After acclimatization, six animals were sampled for gene expression analysis.

2.2. WSSV challenge

After four weeks (28 days) all the animals were challenged by feeding WSSV infected tissue at the rate of 1 g/animal. Sampling was performed at one day intervals for 10 days post-challenge WSSV and the survival was recorded daily. Six animals were sampled at each interval and only those in the intermoult stage were taken for analysis. Mortality by WSSV infection was confirmed by PCR amplification of the WSSV genes and by checking the characteristic white spots on the carapace of infected shrimps.

2.3. Haemolymph collection

Haemolymph was collected from the rostral sinus using capillary tubes (RNase-free) rinsed using pre-cooled anticoagulant solution (RNase free 10% sodium citrate, pH 7.0). Haemolymph was suspended in TRI reagent (Sigma) for total RNA isolation.

2.4. Total RNA isolation and reverse transcription

Total RNA was extracted from the haemocytes using TRI Reagent (Sigma) following manufacturer's protocol. RNA was quantified and checked for purity by spectrophotometry at 260 and 280 nm. Only RNAs with absorbance ratios ($A_{260} : A_{280}$) greater than 1.8 were used for further experiments. First strand cDNA was generated in a 20 μ l reaction volume containing 5 μ g total RNA, 1 \times RT buffer, 2 mM dNTP, 2 μ M oligo d (T_{20}), 20 U of RNase inhibitor and 100 U of M-MLV reverse transcriptase (Fermentas, Inc.). The reaction was conducted at 42 $^{\circ}$ C for 1 h followed by an inactivation step at 85 $^{\circ}$ C for 15 min.

2.5. Semi-quantitative RT-PCR analysis of target gene expression

Time-course analysis of target gene expression was determined by semi-quantitative RT-PCR using elongation factor (ELF) as the internal control. cDNA was diluted 5 times and amplified using Taq polymerase. PCR amplification of 1 μ l of the diluted cDNA was performed in a 25 μ l reaction volume containing 1 \times standard Taq buffer (10 mM Tris-HCl, 50 mM KCl, pH 8.3), 3.5 mM MgCl₂, 200 μ M dNTPs, 0.4 μ M each primer and 1 U Taq DNA polymerase (Fermentas, Inc.).

Amplification was performed using AMP gene primers (ALF, crustin-1, crustin-2, crustin-3, penaeidin-3 and penaeidin-5) (Table 2). Amplification of eight WSSV genes (DNA polymerase, endonuclease, immediate early gene, latency related gene, protein kinase, ribonucleotide reductase, thymidine kinase and VP28) were also analyzed (Table 2). Control genes viz. 18S rRNA, β -actin and ELF were also amplified (Table 2). The thermal profile used was an initial denaturation at 94 $^{\circ}$ C for 2 min followed by 27 cycles of denaturation at 94 $^{\circ}$ C for 15 s,

Table 2
Primers used for the study.

Target gene	Product size	Annealing temp. ($^{\circ}$ C)	Reference
<i>I. Control genes</i>			
18 S rRNA	350 bp	52	Bustin, 2002
β -actin (GQ334394)	520 bp	60	Supungul et al., 2004
ELF (GU732818)	600 bp	60	Loongyai et al., 2007
<i>II. Amp genes</i>			
ALF (GU732817)	300 bp	62	Tharntada et al., 2008
Crustin-1 (GQ334395)	456 bp	55	Supungul et al., 2004
Crustin-2 (FJ535568)	299 bp	60	Chen et al., 2004a
Crustin-3 (GQ334396)	233 bp	60	Jimenez-Vega et al., 2004
Penaeidin-3 (GU732819)	240 bp	60	Jiravanichpaisal et al., 2007
Penaeidin-5 (GQ334397)	300 bp	60	Chen et al., 2004b
<i>III. WSSV genes</i>			
DNA polymerase	586 bp	54	Liu et al., 2005
Endonuclease	408 bp	50	Liu et al., 2005
Immediate early gene	502 bp	54	Liu et al., 2005
Latency related gene	647 bp	53	Liu et al., 2005
Protein kinase	512 bp	55	Liu et al., 2005
Ribonucleotide reductase	408 bp	53	Liu et al., 2005
Thymidine kinase	412 bp	54	Liu et al., 2005
VP 28	555 bp	54	Liu et al., 2005

extension at 68 $^{\circ}$ C for 30 s and a final extension at 68 $^{\circ}$ C for 10 min for the target genes. Annealing temperature varied for the different target genes as given in Table 2. The cycling number of the PCR had been optimized when the target genes and housekeeping genes were amplified at logarithmic phase. The PCR reaction of each sample was carried out in triplicate. PCR product was analyzed by electrophoresis in 1.5% agarose gel in TBE buffer, stained with ethidium bromide and visualized under UV light. The intensity of the gel bands was measured using Image J analysis software.

3. Results

3.1. Expression profile of control genes in the haemocytes of *P. monodon* in response to WSSV challenge

Expression profile of control genes viz. 18S rRNA, β -actin and ELF were found to vary for the various experimental samples analyzed. ELF was found to be the best among the three control genes studied followed by 18S rRNA and β -actin (Fig. 1).

3.2. Expression profile of AMP genes in the haemocytes of *P. monodon* in response to WSSV challenge

All AMP genes viz. ALF, crustin-1, crustin-2, crustin-3, penaeidin-3 and penaeidin-5 were found to be differentially expressed in the haemocytes of *P. monodon* pre- and post-challenge WSSV (Fig. 1). ALF was found to up-regulate post-challenge WSSV. Not much variation in the expression profile of crustin-1 and crustin-2 could be detected in the haemocytes during initial hours of challenge. However, crustin-1 was found to down-regulate considerably during late hours of WSSV infection i.e. from post-challenge day 4 onwards. Expression profile of crustin-3 was worth noticing. This gene was found to up-regulate noticeably post-challenge day 3 to day 10 with considerable expression during late hours of WSSV infection. Penaeidin-3 and penaeidin-5 were found to up-regulate noticeably with WSSV challenge, especially during the 1st two days of WSSV challenge. However, during the late hours of WSSV infection, penaeidins were found to be down-regulated. Generally, ALF and penaeidins (3 and 5) were the only genes found to be up-regulated during early hours of WSSV challenge. In contrast, ALF and crustin-3 were found to be up-regulated considerably during the late hours of WSSV challenge (Fig. 1).

Table 1

Rearing conditions and water quality parameters.

Tank capacity	500 l
Stocking density	12 nos.
Feeding level	4–6% of body weight
Feeding frequency	Twice daily
Water temperature	24–27 $^{\circ}$ C
pH	7.5–8
Salinity	15–18‰
Ammonia	0.01–0.02 mg l ⁻¹
Nitrite	0.00–0.01 mg l ⁻¹
Nitrate	Below detectable level
Alkalinity	50–60
Dissolved oxygen	6–7 mg l ⁻¹

3.3. Expression profile of WSSV genes in the haemocytes of *P. monodon* in response to WSSV challenge

All WSSV gene transcripts could be detected within 48 h of WSSV challenge and revealed an almost similar pattern of expression for all the genes analyzed. None of the WSSV genes could be detected in the shrimp samples pre-challenge confirming the animals used for the study to be free of WSSV infection. Transcripts of DNA polymerase, endonuclease, protein kinase and VP28 genes could be detected post-challenge day 1 onwards. However, immediate early gene, latency related gene, ribonucleotide reductase and thymidine kinase were detected only post-challenge day 2 onwards. Expression of DNA polymerase, ribonucleotide reductase and VP28 genes were very low till 4th day post-challenge WSSV. Then onwards, the amount of all WSSV gene transcripts increased considerably during the experimental period (Fig. 2).

4. Discussion

WSSV is considered to be the most devastating viral disease affecting the shrimp industry around the world (Sanchez-Paz, 2010).

Although extensive research and considerable progress has been made in the characterization of WSSV, little is known about the molecular mechanisms underlying WSSV infection and host immune response. Therefore, a study in this line would be helpful in developing strategies for management of the disease and long-term sustainability of penaeid shrimp farming worldwide.

In the past decade, a number of proteins involved in innate immunity in crustaceans have been characterized at both the protein and molecular level such as members of the proPO activating system, AMPs and lectins (Cerenius and Soderhall, 2004; Gross et al., 2001). However these factors are aimed at bacteria, fungi or parasites rather than viruses. Although AMPs have been well studied in the context of antibacterial and antifungal responses, there is little published data on the possible involvement of AMPs in viral infection (Antony et al., 2011; Dupuy et al., 2004; Liu et al., 2006; Roch et al., 2008). Hence, the present investigation was undertaken to study the expression profile of AMP genes in response to WSSV infection and to examine the antiviral property of AMPs, if any. The transcriptional stabilities of three widely used reference genes, viz. β -actin, ELF and 18S rRNA were also examined (Fig. 1). Analysis of the results proved ELF to be the best

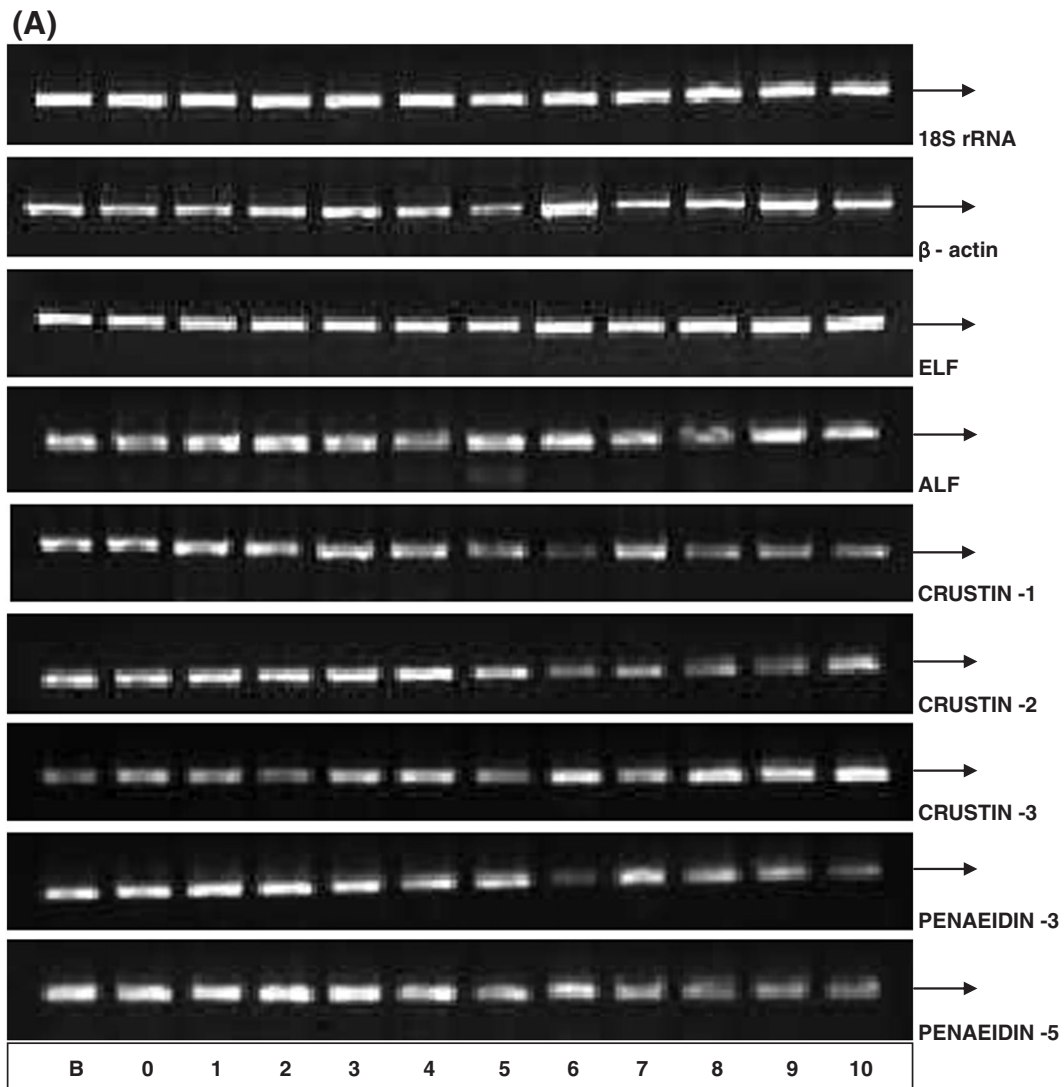


Fig. 1. Time-course analysis of control gene expression (18S rRNA, β -actin, ELF) and AMP gene expression (ALF, crustin-1, crustin-2, crustin-3, penaeidin-3, penaeidin-5) in the haemocytes of giant tiger shrimp, *Penaeus monodon* in response to WSSV challenge. (A) Agarose gel electrophoretogram. (B) Graphical representation of the expression levels of the target genes (x-axis = post-challenge days, y-axis = expression levels of the target genes) (B = Baseline, 0 = pre-challenge WSSV, 1 = 1st day post-challenge WSSV, 2 = 2nd day post-challenge WSSV, 3 = 3rd day post-challenge WSSV, 4 = 4th day post-challenge WSSV, 5 = 5th day post-challenge WSSV, 6 = 6th day post-challenge WSSV, 7 = 7th day post-challenge WSSV, 8 = 8th day post-challenge WSSV, 9 = 9th day post-challenge WSSV, 10 = 10th day post-challenge WSSV).

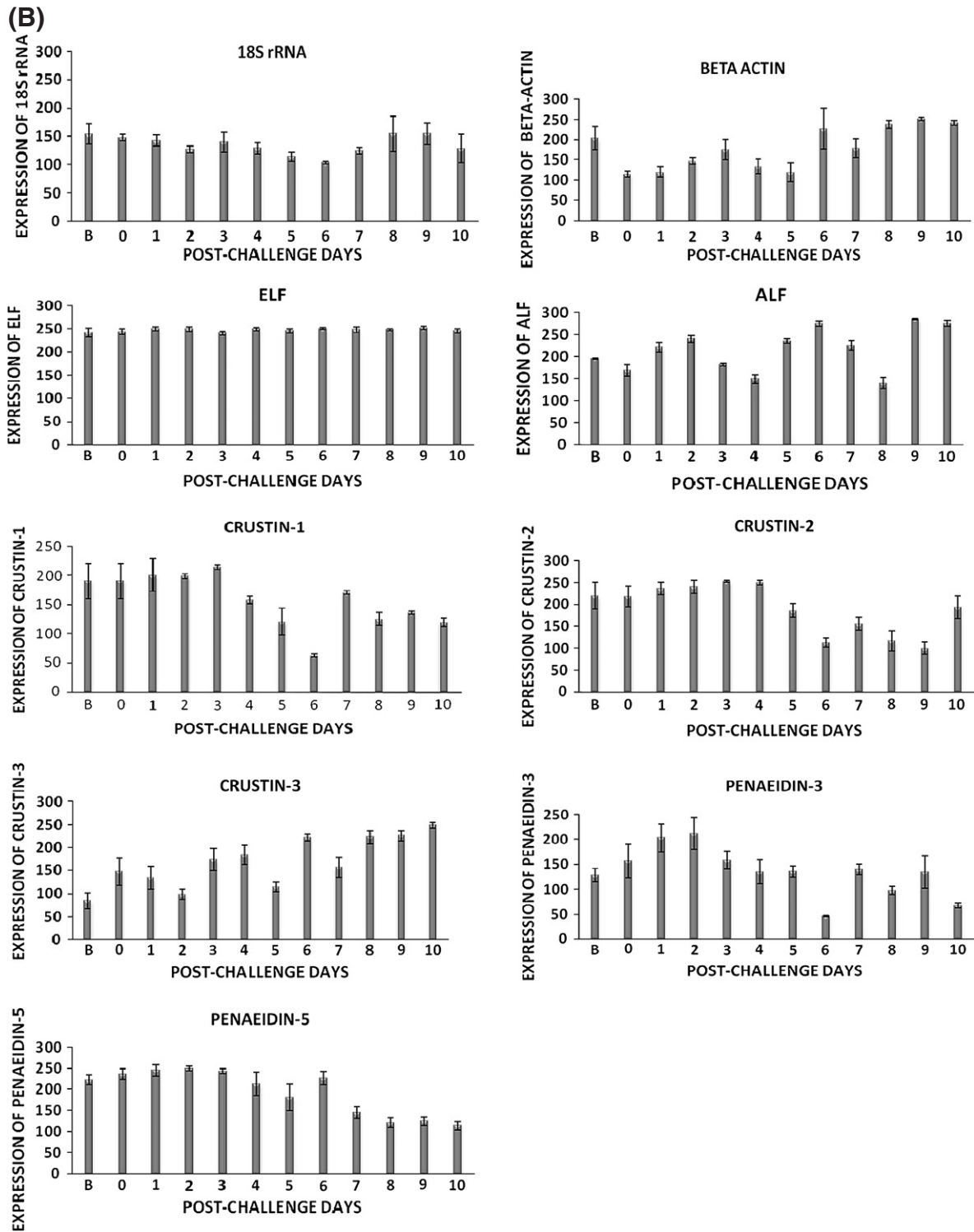


Fig. 1 (continued).

control gene for gene expression studies in shrimps when compared to that of β -actin and 18S rRNA.

AMP genes expressed differentially in the animals both pre and post-challenge (Fig. 1). Considerable expression of crustin-1, crustin-2 and penaeidin-5 could be observed during early hours of WSSV challenge and a noticeable down-regulation during late hours. This might be pointing out to the fact that these molecules are more related to antibacterial and antifungal activities than to antiviral defense. Of particular interest here is the response shown by ALF, crustin-3 and

penaeidins. The results indicated that ALF, crustin-3 and penaeidins respond to WSSV invasion. While both the penaeidins were found to be up-regulated during early hours of WSSV infection; ALF and crustin-3 were found to be up-regulated from early (day 1) to late period (day 10) of WSSV infection. The enhanced production of these AMP transcripts during WSSV infection indicates their possible role in antiviral defense.

ALF, initially characterized in horseshoe crab has been reported to have a specific role in reducing viral replication (Liu et al., 2006). In the

present study, ALF was found to be up-regulated during initial hours of WSSV challenge itself and exhibited an upward trend towards late hours of infection, when other AMP genes down-regulated. Indisputably this sheds light on the involvement of ALF in the host defense mechanisms especially against WSSV. The interference of ALF with viral replication warrants further investigation.

Crustins are cationic, cysteine-rich AMP in crustaceans, with a characteristic WAP domain (Relf et al., 1999). Our identification of crustin-3 over expression in WSSV-challenged *P. monodon* indicates that these molecules might have antiviral activity. Crustin-3 was found to belong to single WAP domain (SWD) crustin type III family. Despite the report of the sequences of several shrimp SWD proteins, no biological function has yet been ascribed to these proteins. Nevertheless, more functional and proteomic studies are needed to elucidate the bioactivity of these molecules.

Penaeidins are the first AMPs to be reported from penaeid shrimps and are characterized by a proline-rich amino terminal region and a cysteine carboxyl-terminal domain (Destoumieux et al., 1997). However, antiviral activities of penaeidins have rarely been investigated. Penaeidins and crustins have been reported to be differentially expressed in the WSSV-infected organisms and are believed to be

directly involved in the immune response of invertebrates (Rojtinnakorn et al., 2002). Our identification of penaeidin-3 over expression in WSSV-challenged *P. monodon* indicates that these molecules might possess antiviral activity. However, the penaeidins got down-regulated during late hours of WSSV infection.

The expression profile of functional and structural genes of WSSV were also analyzed in the present study. All WSSV genes were found to amplify within 48 h of WSSV challenge (Fig. 2). Pattern of expression was almost similar for all the eight WSSV genes studied, except that four of the WSSV genes viz. DNA polymerase, endonuclease, protein kinase and VP28 genes could be detected from 24 h of WSSV challenge; whereas other four WSSV genes viz. immediate early gene, latency related gene, ribonucleotide reductase and thymidine kinase were detected only from 48 h of challenge. The amount of all WSSV gene transcripts were found to increase dramatically from 5th day post-challenge onwards and continued to increase during the experimental period. This shows that WSSV infection has reached its peak from 5th day onwards, when the shrimps were orally challenged with WSSV (Fig. 2). Viral replication could be the predominant process in the haemocytes of WSSV-infected shrimp, allowing them to overcome the expression of shrimp immune related genes.

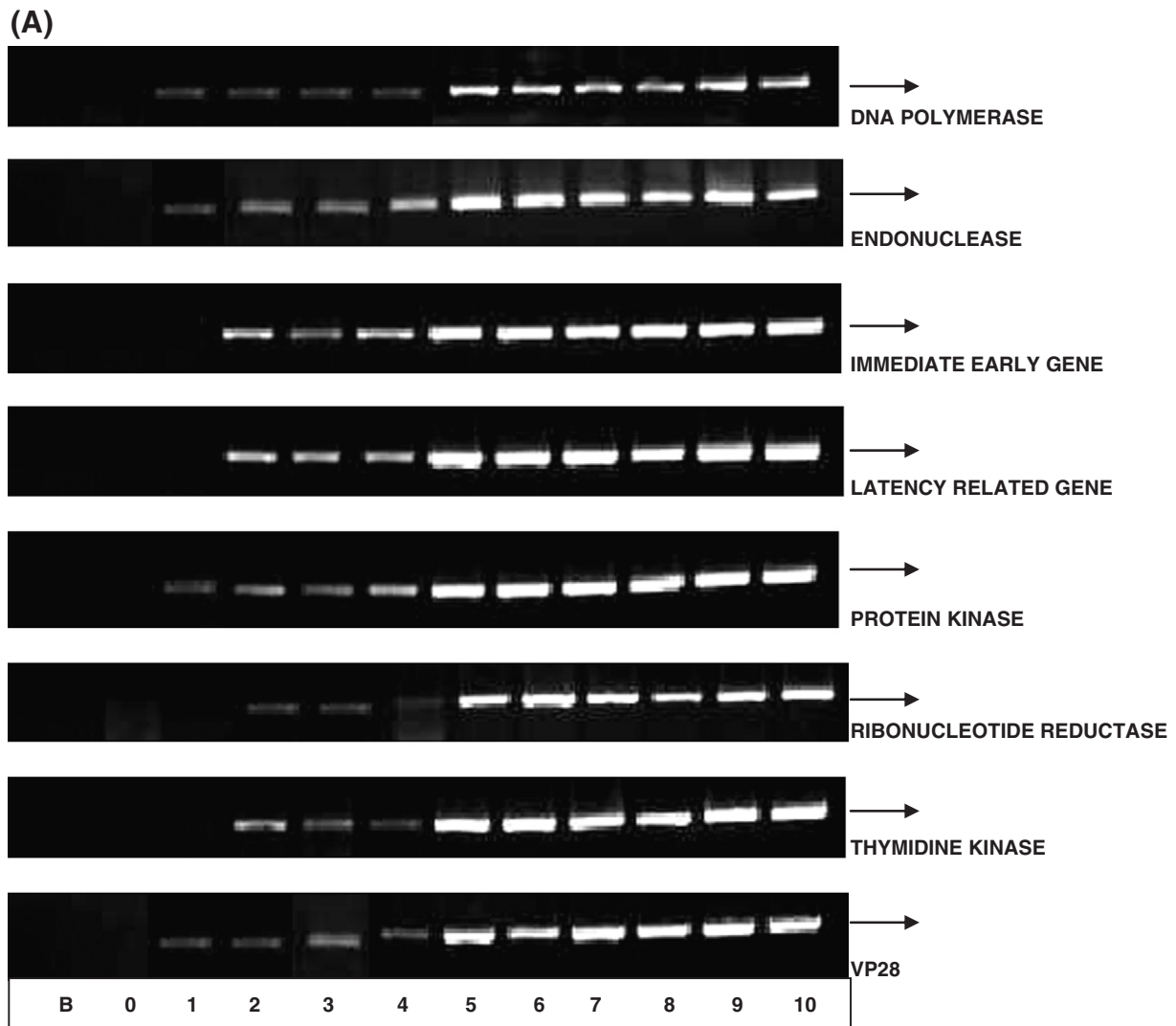


Fig. 2. Time-course analysis of WSSV gene expression (DNA polymerase, endonuclease, immediate early gene, latency related gene, protein kinase, ribonucleotide reductase, thymidine kinase, VP28) in the haemocytes of giant tiger shrimp, *Penaeus monodon* in response to WSSV challenge. (A) Agarose gel electrophoretogram. (B) Graphical representation of the expression levels of WSSV genes (x-axis = post-challenge days, y-axis = expression levels of the WSSV genes) (B = Baseline, 0 = pre challenge WSSV, 1 = 1st day post-challenge WSSV, 2 = 2nd day post-challenge WSSV, 3 = 3rd day post-challenge WSSV, 4 = 4th day post-challenge WSSV, 5 = 5th day post-challenge WSSV, 6 = 6th day post-challenge WSSV, 7 = 7th day post-challenge WSSV, 8 = 8th day post-challenge WSSV, 9 = 9th day post-challenge WSSV, 10 = 10th day post-challenge WSSV).

(B)

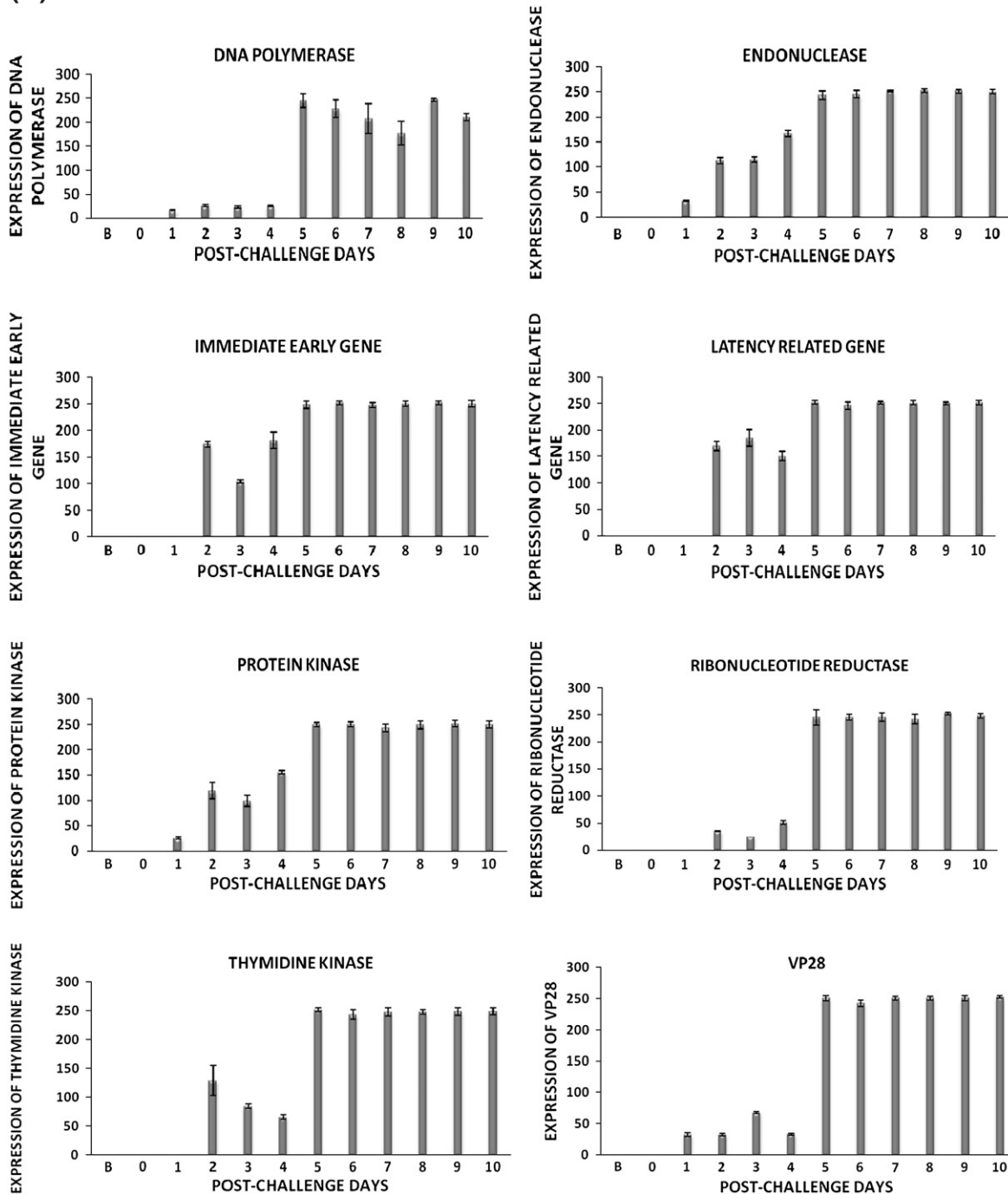


Fig. 2 (continued).

Taken together, our results show that WSSV infection modulates AMP gene expression in haemocytes of WSSV challenged *P. monodon*. The over expression of AMP genes, such as ALF and crustin-3 in WSSV challenged animals, strongly suggests that these genes could be postulated as potential candidates involved in anti-WSSV defense in shrimps. This implies that these peptide molecules are involved in defense mechanisms in *P. monodon* against WSSV invasion. To our knowledge, this is the first report of the expression profile of AMP genes in the haemocytes of WSSV challenged shrimp on a time-course basis. This basic knowledge will provide information on AMP genes involved in shrimp defense against virus invasion. Detailed study on these molecules will allow further exploration of their specific role in

the anti-WSSV defense and hopefully lead to a better understanding of the anti-WSSV peptides and their role in conferring protection to the animals. The information may also be of great value for adopting novel prophylactic strategy in shrimp health management to thwart WSSV infection.

5. Conclusion

Considerable up-regulation in AMP expression could be noticed post-challenge WSSV. A steady increase in ALF and crustin-3 expression could be observed post-challenge WSSV indicating their possible role in antiviral defense. Even though an immediate up-regulation was

noticed for both the penaeidins (3 and 5), their expression was getting decreased progressively as the infection got severe in the animals. The study showed that the WSSV challenge via diet results in shrimp infection at its peak on the fifth day itself and subsequently the animals succumb to death resulting in 100% mortality on 11th day. This information on the differential expression of AMPs would definitely help in formulating a prophylactic strategy in shrimp health management against WSSV by adopting proper probiotics/immunostimulants which will stimulate the expression of these genes thwarting WSSV infection.

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References

- Antony, S.P., Singh, I.S.B., Sudheer, N.S., Vrinda, S., Priyaja, P., Philip, R., 2011. Molecular characterization and expression profile of a crustin-like antimicrobial peptide in the haemocytes of the giant tiger shrimp, *Penaeus monodon*, in response to various immunostimulants and challenge with WSSV. *Immunobiology* 216, 184–194.
- Bustin, S.A., 2002. Quantification of mRNA using real-time reverse transcription PCR-PCR: trends and problems. *J. Mol. Endocrinol.* 29, 23–39.
- Cerenius, L., Soderhall, K., 2004. The prophenol oxidase activating system in invertebrates. *Immunol. Rev.* 198, 72–82.
- Chen, J.Y., Pan, C.Y., Kuo, C.M., 2004a. cDNA sequence encoding an 11.5-kDa antibacterial peptide of the shrimp *Penaeus monodon*. *Fish Shellfish Immunol.* 16, 659–664.
- Chen, J.Y., Pan, C.Y., Kuo, C.M., 2004b. Molecular cloning and sequencing of shrimp *Penaeus monodon* penaeidin-5 cDNA. *Fish Shellfish Immunol.* 16, 665–670.
- Destoumieux, D., Bulet, P., Loew, D., Van Dorsselaer, A., Rodriguez, J., Bachere, E., 1997. Penaeidins, a new family of antimicrobial peptides isolated from the shrimp *Penaeus vannamei* (Decapoda). *J. Biol. Chem.* 272, 28398–28406.
- Dupuy, J.W., Bonami, J.R., Roch, P., 2004. A synthetic antibacterial peptide from *Mytilus galloprovincialis* reduces mortality due to white spot syndrome virus in palaemonid shrimp. *J. Fish Dis.* 27, 57–64.
- Gross, P.S., Bartlett, T.C., Browdy, C.L., Chapman, R.W., Warr, G.W., 2001. Immune gene discovery by expressed sequence tag analysis of hemocytes and hepatopancreas in the Pacific White Shrimp, *Litopenaeus vannamei*, and the Atlantic White Shrimp, *L. setiferus*. *Dev. Comp. Immunol.* 25, 565–577.
- Jimenez-Vega, F., Yepiz-Plascencia, G., Soderhall, K., Vargas-Albores, F., 2004. A single WAP domain-containing protein from *Litopenaeus vannamei* hemocytes. *Biochem. Biophys. Res. Commun.* 314, 681–687.
- Jiravanichpaisal, P., Puanglarp, N., Petkon, S., Donnuea, S., Soderhall, I., Soderhall, K., 2007. Expression of immune-related genes in larval stages of the giant tiger shrimp, *Penaeus monodon*. *Fish Shellfish Immunol.* 23, 815–824.
- Liu, W.J., Chang, Y.S., Wang, C.H., Kou, G.H., Lo, C.F., 2005. Microarray and RT-PCR screening for white spot syndrome virus immediate early genes in cycloheximide-treated shrimp. *Virology* 334, 327–341.
- Liu, H., Jiravanichpaisal, P., Soderhall, I., Cerenius, L., Soderhall, K., 2006. Antilipopolysaccharide factor interferes with white spot syndrome virus replication *in vitro* and *in vivo* in the crayfish *Pacifastacus leniusculus*. *J. Virol.* 80, 10365–10371.
- Loongyai, W., Avarre, J.C., Cerutti, M., Lubzens, E., Chotigeat, W., 2007. Isolation and functional characterization of a new shrimp ovarian peritrophin with antimicrobial activity from *Fenneropenaeus merguensis*. *Mar. Biotechnol.* 95, 624–637.
- Relf, J.M., Chisholm, J.R., Kemp, G.D., Smith, V.J., 1999. Purification and characterization of a cysteine-rich 11.5-kDa antibacterial protein from the granular haemocytes of the shore crab, *Carcinus maenas*. *Eur. J. Biochem.* 264, 350–357.
- Roch, P., Yang, Y., Toubiana, M., Aumelas, A., 2008. NMR structure of mussel mytilin, and antiviral-antibacterial activities of derived synthetic peptides. *Dev. Comp. Immunol.* 32, 227–238.
- Rojtinnakorn, J., Hirono, I., Itami, T., Takahashi, Y., Aoki, T., 2002. Gene expression in haemocytes of kuruma prawn, *Penaeus japonicus*, in response to infection with WSSV by EST approach. *Fish Shellfish Immunol.* 13, 69–83.
- Rosa, R.D., Barracco, M.A., 2010. Antimicrobial peptides in crustaceans. *ISJ* 7, 262–284.
- Sanchez-Paz, A., 2010. White Spot Syndrome Virus: an overview on an emergent concern. *Vet. Res.* 41, 43.
- Soderhall, K., Cerenius, L., 1992. Crustacean immunity. *Annu. Rev. Fish Dis.* 2, 3–23.
- Supungul, P., Klinbunga, S., Pichyangkura, R., Hirono, I., Aoki, T., Tassanakajon, A., 2004. Antimicrobial peptides discovered in the black tiger shrimp *Penaeus monodon* using the EST approach. *Dis. Aquat. Organ.* 6, 123–135.
- Tharntada, S., Somboonwiwat, K., Rimphanitchayakit, V., Tassanakajon, A., 2008. Antilipopolysaccharide factors from the black tiger shrimp, *Penaeus monodon*, are encoded by two genomic loci. *Fish Shellfish Immunol.* 24, 46–54.
- Tincu, A.J., Taylor, S.W., 2004. Antimicrobial peptides from marine invertebrates. *Antimicrob. Agents Chemother.* 48, 3645–3654.
- Wongpanya, R., Aoki, T., Hirono, I., Yasuike, M., Tassanakajon, A., 2007. Analysis of gene expression in haemocytes of shrimp *Penaeus monodon* challenged with White Spot Syndrome virus by cDNA microarray. *Sci. Asia* 33, 165–174.