Fish and Shellfish Immunology 87 (2019) 755–764

Contents lists available at ScienceDirect

Fish and Shellfish Immunology

journal homepage: www.elsevier.com/locate/fsi

Full length article

Comparative transcriptomic analysis of *Marsupenaeus japonicus* hepatopancreas in response to *Vibrio parahaemolyticus* and white spot syndrome virus

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ARTICLE INFO

Keywords:
Vibrio parahaemolyticus
White spot syndrome virus
Immune response
Marsupenaeus japonicus
Transcriptomic analysis
Next-generation sequencing

ABSTRACT

Vibrio parahaemolyticus and white spot syndrome virus (WSSV) are pathogens that cause epidemics in kuruma shrimp (*Marsupenaeus japonicus*) during aquaculture, resulting in severe economic losses to local farmers. To characterise the mechanisms of the molecular responses to *V. parahaemolyticus* and WSSV infection in *M. japonicus*, the transcriptome of hepatopancreas was sequenced using next-generation sequencing after infection. A total of 29,180 unigenes were assembled, with an average length of 1,151 bp (N50 = 1,951 bp). After BLASTX searching against the Nr database (E-value cut-off = 10^{-5}), 15,176 assembled unigenes remained, with 3,039 and 1,803 differentially expressed transcripts identified in the *V. parahaemolyticus*- and WSSV-infected groups, respectively. Of these, 1466 transcripts were up-regulated and 1573 were down-regulated in *V. parahaemolyticus*-infected shrimps, and 970 transcripts were up-regulated and 833 were down-regulated in the WSSV-infected shrimps. Additionally, 761 transcripts were differentially expressed in both *V. parahaemolyticus*- and WSSV-infected shrimps. Several known immune-related genes including *caspase 4*, *integrin*, *crustin*, *ubiquitin-conjugating enzyme E2*, *C-type lectin*, and α_2*-macroglobulin* were among the differentially expressed transcripts. These results provide valuable information for characterising the immune mechanisms of the shrimp responses of to *V. parahaemolyticus* andWSSV infection.

1. Introduction

Kuruma shrimp (*Marsupenaeus japonicus*) is one of the most commercially important crustacean species in the fisheries and aquaculture industry of the Indo-West Pacific region [1]. Owing to its economic value, the kuruma shrimp industry is now expanding rapidly in China. In 2017 alone, the culture area of this shrimp was 1,716 ha, and the yield was 52,466 tons [2]. However, this industry has witnessed huge economic losses due of intensive farming methods that have led to the spread of several serious bacterial and viral diseases [3], among which Gram-negative bacterium *Vibrio parahaemolyticus* is highly prevalent [4]. *V. parahaemolyticus* is a common bacterial pathogen of shrimps, and is thought to be the cause of early mortality syndrome, also known as acute hepatopancreatic necrosis disease (AHPND) [5]. White spot syndrome virus (WSSV) is the most serious viral pathogen of shrimps, and can cause cumulative mortality of up to 100% within 3–10 days [6]. Shrimp disease control and prevention are therefore crucial areas of research, and understanding the immune regulation mechanisms of shrimps against invading pathogens could assist strategies for preventiing and treating these and other diseases.

Like other invertebrates, crustaceans mainly rely on the innate immune system, which consists of humoral and cellular responses against viral infections [7]. The innate immune response is the first line of defence against viral infections. It triggers diverse activities via signal transduction, including Toll, IMD, and JAK-STAT pathways, and integrin-mediated signal transduction [8]. Cellular immune reactions, including phagocytosis, apoptosis, and encapsulation, as well as the humoral responses involve the synthesis and release of several immune proteins, such as antimicrobial peptides, proteinase inhibitors, and cytokine-like factors [9].

In crustaceans, in addition to functioning as a digestive gland, the hepatopancreas is also an important organ for immunity [10]. Epithelial cells of the hepatopancreas are major sources of immune response molecules including lectins, hemocyanin, ferritin, antibacterial and

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https://doi.org/10.1016/j.fsi.2019.02.030
Received 12 December 2018; Received in revised form 13 February 2019; Accepted 15 February 2019

Abbreviations

WSSV	white spot syndrome virus
APND	acute hepatopancreatic necrosis disease
RNA-Seq	RNA sequencing
KEGG	Kyoto Encyclopedia of Genes and Genomes
KOG	Cluster of Orthologous Groups
DEGs	differentially expressed genes
LGBP	beta-1,3-glucan binding protein
α_2M	alpha 2 macroglobulin
PRPs	pattern recognition proteins

antiviral proteins, proteolytic enzymes, and nitric oxide [11]. Large-scale identification of immunity-related genes from the hepatopancreas is of great value for studying immune mechanisms in crustaceans. However, the genome sequence is unavailable for many shrimp species, limiting available molecular information. High-throughput RNA sequencing (RNA-Seq) is a highly effective technology for analysis of gene expression, discovery of novel transcripts, and identification of differentially expressed genes (DEGs) [12], and can be readlily applied to species lacking complete genome sequences and non-model organisms [13]. Indeed, RNA-Seq has been used to investigate immune reactions against viruses in Chinese shrimp (*Fenneropenaeus chinensis*) afflicted with acute WSSV infection [14,15].

Many immunity molecules, including α_2-macroglobulin (α_2M) [16], MnSOD [17], Toll receptor [18], and β-1, 3-glucan-binding protein [19] have been isolated and characterised. However, there is limited knowledge regarding the antiviral immunity system of kuruma shrimp, and the signalling pathways implicated in antiviral immunity responses also remain poorly understood. In the present study, we obtained a transcriptome data from the hepatopancreas of kuruma shrimp experimentally infected with WSSV or *V. parahaemolyticus*, and analysed the data using Illumina sequencing and bioinformatics. The main aim of this study was to identify candidate proteolytic cascades and signal transduction pathways involved in immune defences against WSSV infection at the transcriptome level, which may provide insight into shrimp antiviral immunity and shrimp-virus interactions. These results could assist the development of disease control strategies for aquaculture of kuruma shrimp.

Table 1
Primers used for qPCR analysis.

Gene	Sequence (5' – 3′)	Annealing temperature (°C)
Caspase 4	GTGGCGAGTGGAGGTCAGTA GTTTGTCTGTCTGCCTATGG	57.5
Integrin	ATCCTGGTCAAAGACGCACATAA CGCTCATCAGGGGCATCATC	58.6
Crustin	TGCTCAGAACTCCCTCCACC TTGAATCAGCCCATCGTCG	57.7
Ubiquitin-conjugating enzyme E2	CATCAGCACACAAGGAACTATT CGTCCATCTACGAGCATTCT	57.8
C-type lectin	TTCTCAAGGCTACACTGGACG CCTTCTGCGTTATCAGGTTGTT	57.2
α_2 *-Macroglobulin*	CAAGGACCTCTGGATGTCGTAGC GTTGACCTCGGTTGGGAAAGT	58.5
EF1-a	GGAACTGGAGGCAGGACC AGCCACCGTTTGCTTCAT	58.0

Table 2
General transcriptome data information for *V. parahaemolyticus*-infected, WSSV-infected, and control shrimp groups.

Sample	Total number	Total length	Mean length	N50	N70	N90	GC (%)
Control_1	21,635	20,385,783	942	1,619	914	367	43.39
Control_2	22,449	20,934,866	932	1,588	904	363	43.36
Control_3	20,037	16,835,686	840	1,381	777	333	43.4
VP_1	19,710	17,395,073	882	1,463	838	353	43.02
VP_2	20,859	18,340,516	879	1,474	832	348	43.04
VP_3	21,708	20,506,606	944	1,621	929	373	43.21
WSSV_1	17,449	14,456,436	828	1,364	766	330	43.69
WSSV_2	19,479	16,460,383	845	1,405	789	332	43.49
WSSV_3	18,059	15,190,389	841	1,373	781	339	43.13
All-Unigene	29,180	33,609,331	1,151	1,951	1,225	474	43.39

2. Materials and methods

2.1. Shrimp culture and pathogen infection

M. japonicus (body weight 8.6 ± 1.2 g) were purchased from a commercial aquaculture market in Qingdao, Shandong Province, China. Shrimps were acclimatised for 1 week in tanks (300 L) with aerated and filtered freshwater at 27 ± 1 °C in the laboratory. They were then randomly sampled and tested by PCR to ensure they were free from *V. parahaemolyticus* and WSSV. They were then divided into three groups; a *V. parahaemolyticus*-infected group, a WSSV-infected group, and a negative control group. For the *V. parahaemolyticus*-infected group, each shrimp was intramuscularly injected with 100 μL of filtered supernatant obtained from *M. japonicus* with AHPND at a cell density of 10^6 colony-forming units (cfu)/mL. Similarly, animals in the WSSV-infected group were injected with 100 μL of filtered supernatant obtained from WSSV-infected *M. japonicus* (10^7 copies/mL, identified by qPCR) [20,21]. The control group received 100 μL of phosphate-buffered saline (PBS, pH 7.4) per individual. Ten individuals from each group were randomly selected at 24 h post-infection, and hepatopancreas tissue was dissected and preserved in liquid nitrogen until RNA extraction. Three biological replicates were performed in the three experiment groups, receiving a total of nine samples.

2.2. RNA extraction and transcriptome sequencing

Total RNA was isolated from hepatopancreas tissue using sqRT-PCR RNAiso Plus (TaKaRa, Dalian, China). cDNA was synthesiSed using the Perfect Real Time version of the PrimerScript RT reagent kit with gDNA Eraser (TaKara) according to the manufacturer's instructions. Next, RT-PCR was performed to analyse genes in a total reaction volume of 25 μL, according to the manufacturers' instructions.

For Illumina paired-end sequencing, equivalent quantities of total RNA isolated from three shrimp were pooled as one sample and three samples were included in each group. After poly (A) mRNA was purified and fragmented into small pieces, we used random hexamer primers and reverse transcriptase (Invitrogen) to carry out first-strand cDNA synthesis. Second-strand cDNA synthesis was then performed with RNase H (Invitrogen) and DNA polymerase I (New England BioLabs, Beijing, China). A cDNA library was constructed with average insert sizes of 200–500 bp and cDNA sequencing was performed using an Illumina HiSeq 4000 system according to the manufacturer's protocol, with read lengths of 150 bp in transcriptome quantification analysis. Three independent cDNA libraries were constructed in parallel, according to the transcriptome protocol. Transcriptome sequencing was performed by BGI (Shenzhen, China).

2.3. Analysis of differentially expressed genes

The annotated genome of *M. japonicus* has not been published; therefore, *de novo* assembly was used as a reference for further analysis.

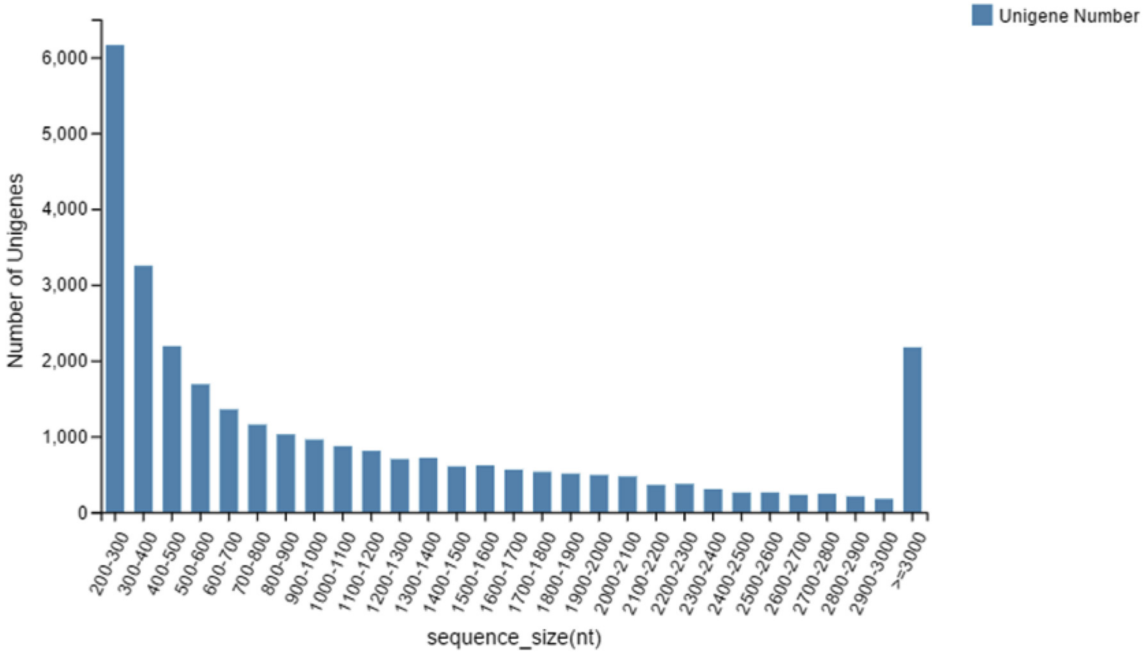

Fig. 1. Length distribution of *M. japonicus* hepatopancreas transcripts.

Table 3
Annotation of unigenes from *M. japonicus* hepatopancreas transcriptome.

Values	Total	NR	NT	Swissprot	KEGG	KOG	Pfam	GO	Overall
Number	29,180	15,176	8,466	12,830	12,927	12,093	12,223	4,525	17,870
Percentage	100%	52.01%	29.01%	43.97%	44.30%	41.44%	41.89%	15.51%	61.24%

Firstly, raw reads were filtered to remove adaptor and low quality sequences (with a quality score less than 20). After filtering, clean reads were assembled into unigenes using Trinity *de novo* assembler, followed by the TGICL clustering tool. The reads from the control, *V. parahaemolyticus*, and WSSV treatment groups were mapped against the assembled unigenes using HISAT. The FPKM (fragments per kb per million reads) method was used to calculate the expression abundance. Each unigene was subjected to a BLASTx search against the NCBI non-redundant protein database with an e-value threshold of 10^{-3}. The R package DESeq2 was used to identify the DEGs. A DEG was considered as an unigene with greater than 2-fold change and p-value < 0.05. Gene Ontology (GO) terms and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway annotation were achieved using the Blast2GO program and KAAS (KEGG Automatic Annotation Server) on-line program (http://www.genome.jp/kaas-bin/kaas_main), respectively.

2.4. Validation of differentially expression genes by qRT-PCR

To validate the transcriptome data, 1 μg of the high quality hepatopancreas RNA samples, extracted using the mirVana miRNA Isolation Kit (Ambion, Austin, TX, USA) was reverse-transcribed using the Prime Script RT Reagent Kit with gDNA Eraser (TaKaRa) following the manufacturer's protocol. Six significantly up-regulated genes including two genes (*Caspase 4* and *Integin*) were expressed only in *V. parahaemolyticus*-infected group, two genes (*Crustin* and *Ubiquitin-conjugating enzyme E2*) were expressed in both *V. parahaemolyticus* and WSSV-infected groups, and two genes (*C-type lectin* and *α_2-Macroglobulin*) were expressed only in WSSV-infected group were selected for validation by qRT-PCR using a Light Cycler 480 RT-PCR system (Roche Applied Science, Switzerland) with an initial denaturation step at 95 °C for 10 min, followed by 45 cycles at 95 °C for 15 s and 60 °C for 30 s. Each qPCR assay was carried out in triplicate for each sample, and five shrimps were analysed in each group. Fold changes in gene expression were computed using the relative quantification method and normalised against *M. japonicus EF1α* expression. Gene-specific qRT-PCR primers designed using Primer 5 software are listed in Table 1.

2.5. Statistical analysis

All data are presented as means ± standard deviation (SD). Data normality was checked by Shapiro-Wilk tests. One-way analysis of variance (ANOVA) and multiple comparison Tukey's tests were used to compare significant differences using the SPSS 19.0 program (SPSS Inc., Chicago, IL, USA). A *p*-value < 0.05 was considered statistically significant.

3. Results

3.1. De novo assembly and annotation of unigenes

Comparison with GenBank protein and nucleotide sequences identified 29,180 unigenes as previously known genes, with an average length of 1,151 bp (Table 2). The length of unigenes ranged from 300 nt to ≥3,000 nt (Fig. 1). All unigenes were annotated using BLASTx with protein databases Nr, Swiss-Prot, KEGG, Cluster of Orthologous Groups (KOG), and GO, and BLASTn with nucleotide databases (Nt), resulting in 26,634 unigenes assigned, representing ~61.24% unigens (Table 3).

Through GO annotation, 7,124 unigenes from the assembly were assigned GO terms for biological process (18 subcategories), cellular component (14 subcategories), and molecular function (12 subcategories). The major GO term subcategories (i.e., representing the top 30% of unigenes) were cellular process, cell, cell part, metabolic process, binding, single-organism process, catalytic activity, organelle, and

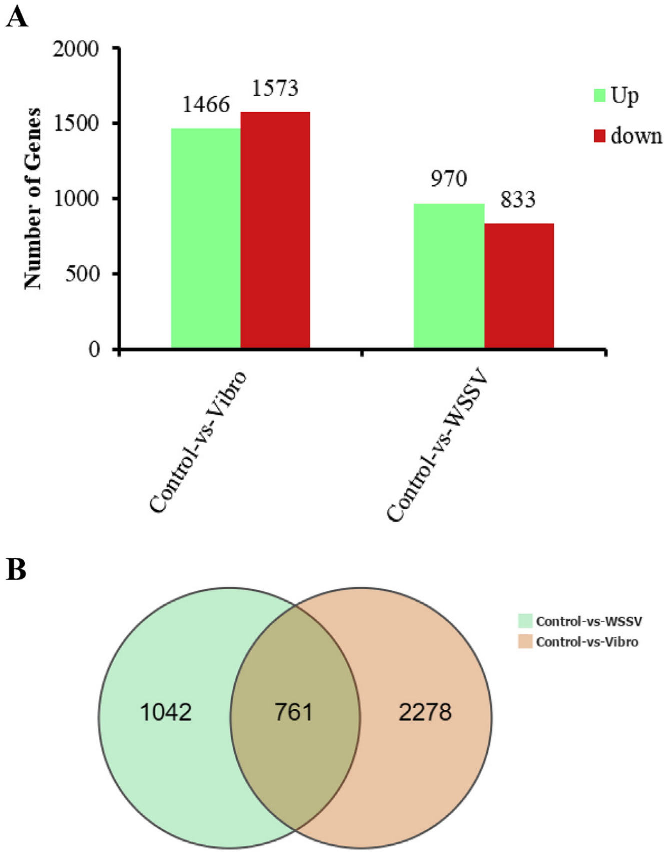

Fig. 2. Bioinformatics analysis of differentially expressed transcripts (A) Numbers represent up- and down-regulated transcripts (> 2-fold) compared with the control group. Venn diagram of differentially expressed transcripts (B). Numbers represented up- and down-regulated transcripts (> 2-fold) compared with the control group.

biological regulation (Fig. S1A). Using the KOG database to further explore protein orthologs of the assembled unigenes, 12,093 were successfully annotated with 26 specific protein function definitions or orthologous categories (Fig. S1B). Among these protein function categories, the largest four were general function prediction only (5,753), translation, ribosomal structure and biogenesis (3,605), transcription (2,684), and replication, recombination, and repair (2,340). To identify the biological processes of the annotated unigenes, KEGG pathway analysis was performed, and 18,599 unigenes were annotated into six major KEGG pathways; cellular processes, environmental information processing, genetic information processing, human diseases, metabolism, and organismal systems. These annotated unigenes were further divided into 42 level 2 subcategories, except for global and overview maps that contained no pictorial information. The largest subcategory group, signal transduction, included 1,556 annotated genes, followed by transport and catabolism (1,183) and immune system (1,036; Fig. S1C).

3.2. Classification and analysis of differentially expressed genes

To furthor analyse and characterise the DEGs, a false discovery rate (FDR) cut-off of ≤ 0.05 and a $\log_2$ ratio ≥ 2 were exployed as thresholds for selecting DEGs. Following *V. parahaemolyticus* infection, 1,466 genes were up-regulated, and 1,573 genes were down-regulated. After WSSV injection, 970 genes were up-regulated, and 833 genes were down-regulated (Fig. 2A). Interestingly, among these DEGs, 761 were significantly up-regulated or down-regulated during both *V. parahaemolyticus* and WSSV infection (Fig. 2B).

3.3. GO and KEGG analysis of differentially expressed genes

Unigenes in the *V. parahaemolyticus*-infected group were aligned to GO terms (Fig. 3A). The 267 unigenes expressed in the *V. parahaemolyticus*-infected group were mostly divided into three categories with 57 functional groups, consisting of biological process (20 functional groups), cellular component (17 functional groups), and molecular function (10 functional groups). KEGG pathway classification of DEGs (1129). indicated that more unigenes (671)) were assicuated with signal transduction pathways than any other pathway (Fig. 3B). Metabolism was a crucial pathway within the larger category.

Unigenes in the WSSV-infected group were aligned to GO terms (Fig. 4A). The 267 unigenes expressed in the WSSV-infected group were mainly divided into three categories with 57 functional groups consisting of biological process (20 functional groups), cellular component (17 functional groups), and molecular function (10 functional groups). KEGG pathway classification was performed on DEGs (9,374), and more transcripts (793) were associated with signal transduction pathways than any other pathways (Fig. 4B). Metabolism was a crucial pathway within the larger category.

3.4. GO and KEGG analysis of 761 transcripts

We used 761 differentially expressed transcripts for GO classification (Fig. 5A). In terms of biological process, the major functions were transcription and regulation of transcription. In terms of cellular component classification, transcripts were overrepresented in the nucleus, integral membrane, and cytoplasm categories. In terms of molecular function, they were overrepresented in ATP binding and zinc ion binding. KEGG pathway classification was subsequently performed to analyse the 761 DEGs (Fig. 5B).

3.5. Potential immunity-related genes in M. japonicas

Many of the aberrantly expressed genes found in the *V. parahaemolyticus*- and WSSV-infected groups are known to be linked to various processes clustered under the animal immune system. There were 131 immunity genes specifically expressed in the *V. parahaemolyticus*-infected group, and these were grouped into 18 functions, including antimicrobial proteins, proteases and proteinases, and signal transduction (Table S1). There were 49 genes linked to immunity function specifically expressed in WSSV-infected shrimps, grouped into 12 functions (Table S2). Among the 761 transcripts common to both *V. parahaemolyticus*- and WSSV-infected groups, 47 immune genes were identified, and were grouped into 11 functions (Table S3).

3.6. Validation of RNA-seq transcriptome data by qRT-PCR

To verify the transcriptome data and to better understand the responses of the shrimp hepatopancreas to *V. parahaemolyticus* and WSSV infection, qRT-PCR was performed. As mentioned above, DEGs were categorised using KEGG pathway enrichment analysis (Figs. 3–5b). We found that genes involved in cell growth and anti-apoptosis were strongly induced in the transcriptomes of the *V. parahaemolyticus*- and WSSV-infected groups compared with the control group. Six genes (*caspase 4*, *integrin*, *crustin*, *ubiquitin-conjugating enzyme E2*, *C-type lectin*, and *α_2M*) were selected for validation using qPCR (Fig. 6). The qPCR results showed a significant up-regulation in the expression of *caspase 4* and *integrin* in the *V. parahaemolyticus*-infected group compared with the WSSV-infected and control groups. A significant up-regulation in the expression of *crustin* and *ubiquitin-conjugating enzyme E2* were observed in both *V. parahaemolyticus*- and WSSV-infected groups compared with the control group. A significant up-regulation was observed in the expression of *C-type lectin* and the α_2M isoform in the WSSV-infected group, while there was no significant change in expression of this gene in the *V. parahaemolyticus*-infected group compared with the

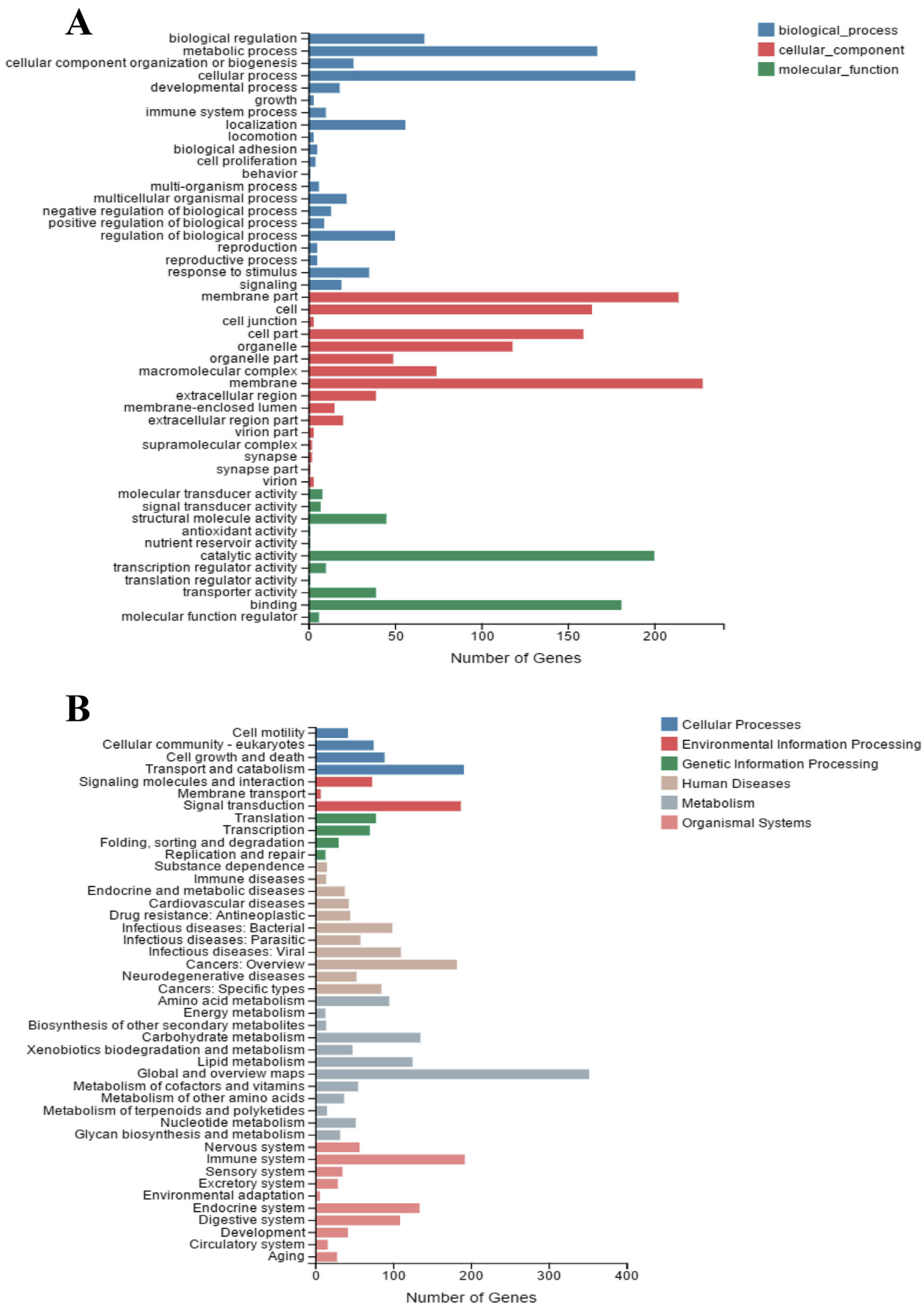

Fig. 3. Analysis of differentially expressed genes (DEGs) between control and *V. parahaemolyticus* -infected groups (excluding the 761 transcripts differentially expressed in both *V. parahaemolyticus* and WSSV infectios). (A) Analysis of Gene Ontology (GO) term functional enrichment. (B) KEGG pathway enrichment analysis of DEGs.

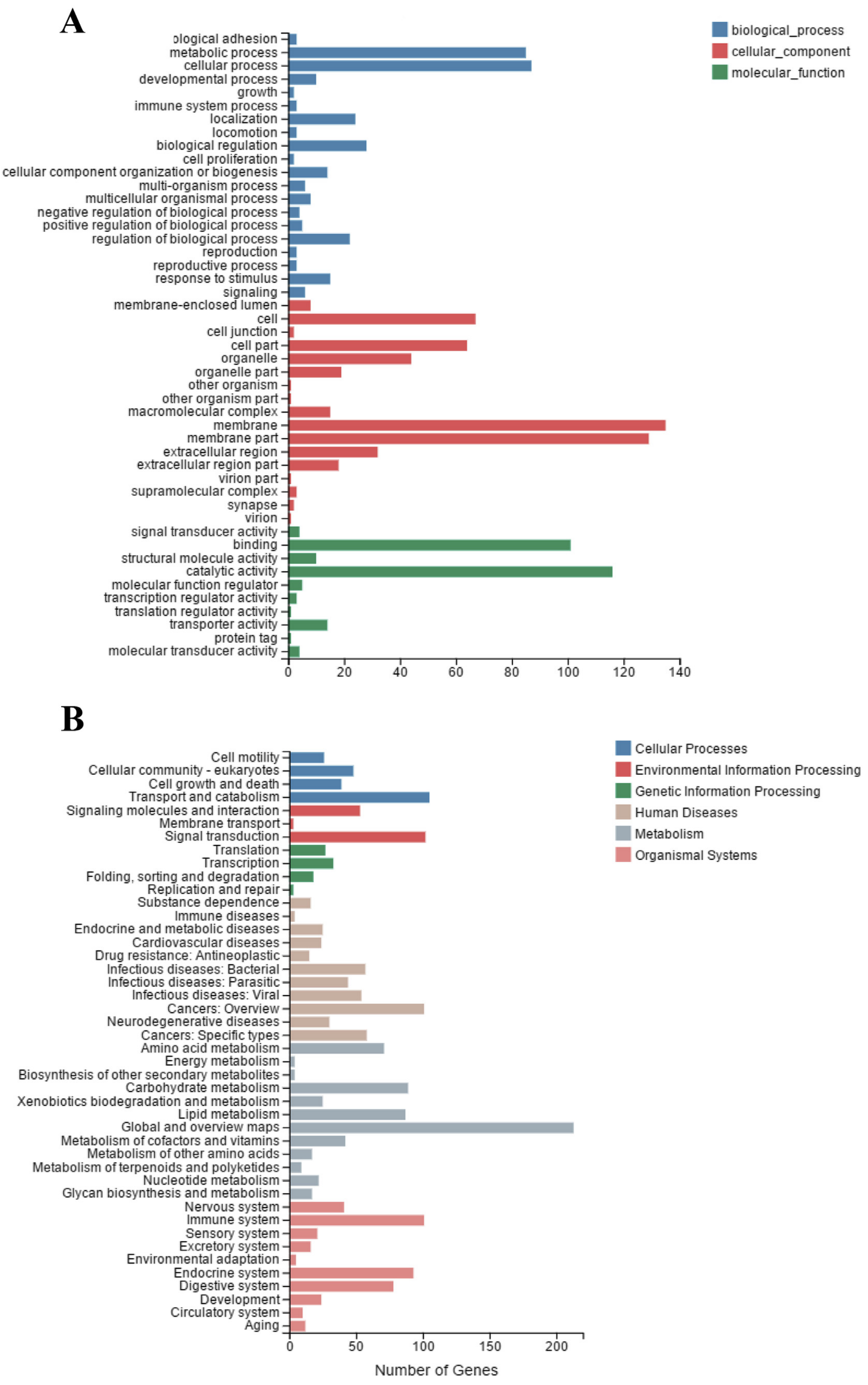

Fig. 4. Analysis of DEGs between control and WSSV-infected groups (excluding the 761 transcripts differentiallyexpressed in both *V. parahaemolyticus* and WSSV infections). (A) Analysis of GO term functional enrichment. (B) KEGG pathway enrichment analysis.

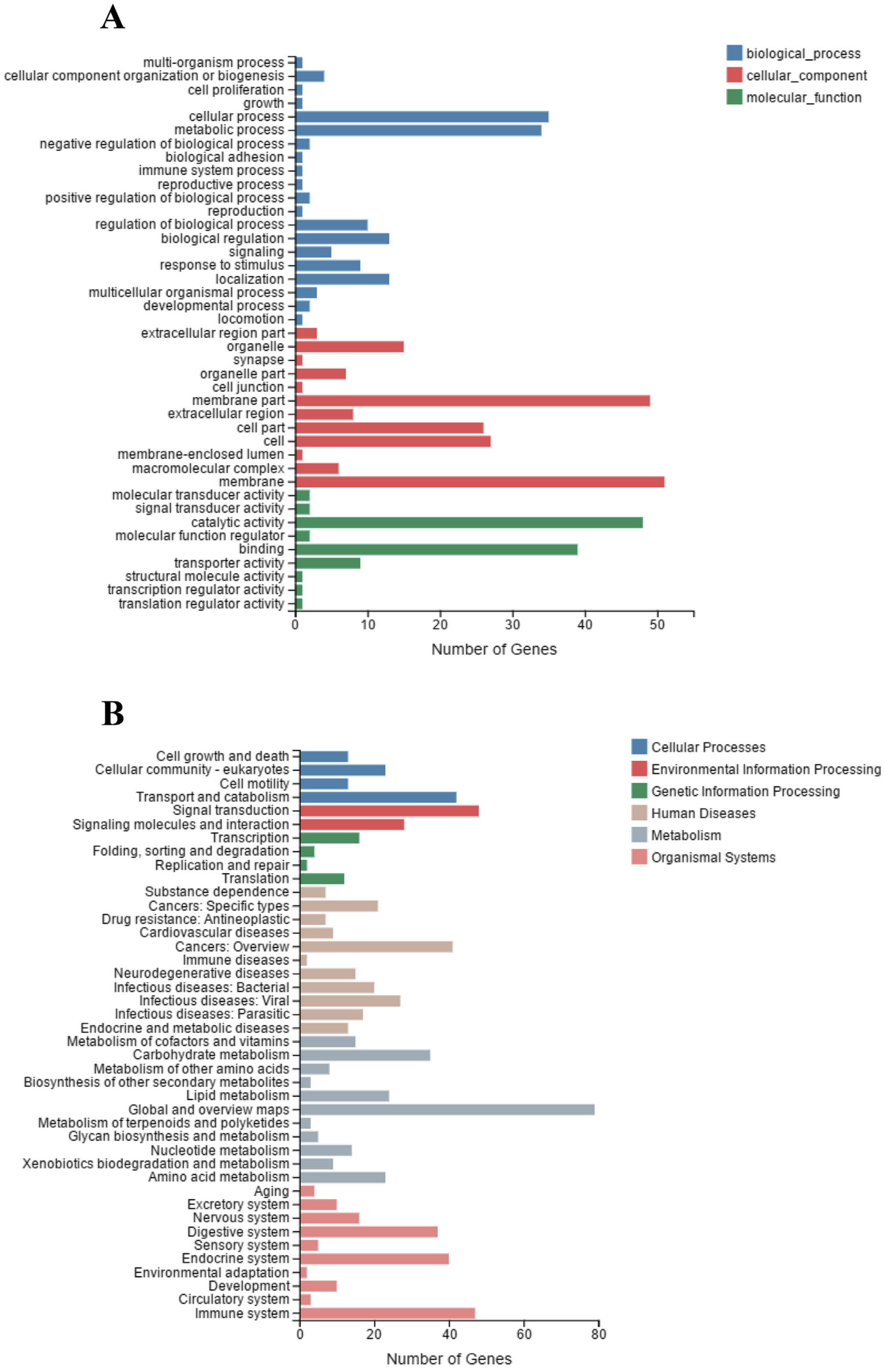

Fig. 5. Analysis of the 761 transcripts differentially expressed in both *V. parahaemolyticus*-infected and the WSSV-infected shrimps (A) Analysis of GO term functional enrichment (B) KEGG pathway enrichment analysis.

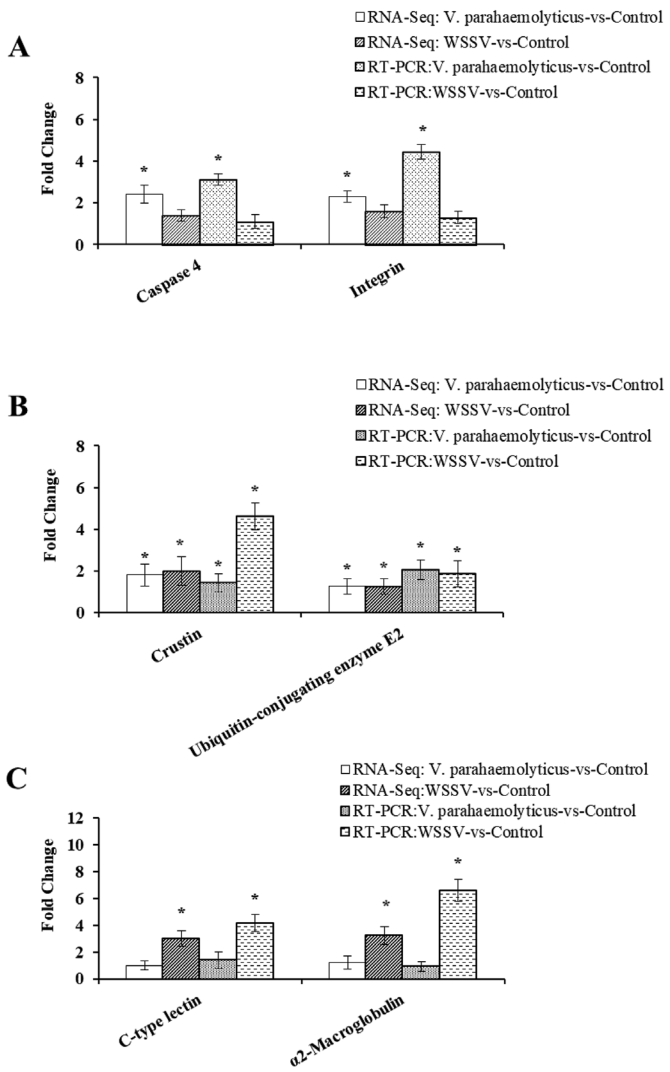

Fig. 6. Validation of selected DEGs by qPCR. To validate RNA-seq data, the relative gene expression levels of six selected DEGs; (A) *Caspase 4* and *Integin* were expressed only in *V. parahaemolyticus*-infected group, (B) *Crustin* and *Ubiquitin-conjugating enzyme E2* were expressed in both *V. parahaemolyticus* and WSSV-infected groups, (C) *C-type lectin and α_2-Macroglobulin* were expressed only in WSSV-infected group were examined by qPCR. *EF1a* was used as an internal control. Asterisk on the graph denotes statistically differences ($p < 0.05$).

control group.

4. Discussion

The recently emerged shrimp disease AHPND can cause mass mortality (up to 100%) and is known to be caused by a unique strain of *V. parahaemolyticus* carrying toxin-producing plasmids [22]. *V. parahaemolyticus* first infects the stomach, then releases pirA- and pirB-like toxins in the hepatopancreas. The released toxins cause severe cellular damage and necrosis of the hepatopancreas [23]. The shrimp immune defence mechanisms triggered in response to AHPND remain unclear.

WSSV is one of the most devastating viral diseases in shrimps, causing high mortality and severe economic losses in the shrimp farming industry throughout the world [24]. Shrimps such as *M. japonicus* rely on the innate immune system to defend themselves against invading microbes by recognising and clearing them through humoral and cellular immune responses [25,26]. The hepatopancreas of *M. japonicus* is the primary production site for two classes of pattern recognition proteins, beta-1,3-glucan binding protein (LGBP) and C-type lectins [27]. LGBP functions to recognise and eliminate pathogens, whereas C-type lectins facilitate recognition and pathogen phagocytosis through opsonisation [28]. Transcriptome sequencing is a powerful tool to better understand the pathways and genes involved in immune responses to infections [29].

In the present study, we acquired transcriptome profile data for *V. parahaemolyticus*- and WSSV-infected *M. japonicus*, and carried out a comparative analysis. We found that *V. parahaemolyticus* infection significantly affected the expression of more transcripts in the hepatopancreas than did WSSV infection, consistent with previous studies [15]. Many DEGs between *V. parahaemolyticus*- and WSSV-infected group potentially involved in immunity were identified. The main difference between groups was the up- and down-regulation of genes; 2,278 genes were significantly up- or down-regulated during *V. parahaemolyticus* infection, compared with 1,042 genes during WSSV infection, and 761 genes were altered in both *V. parahaemolyticus* and WSSV infections. The 2,278 DEGs between the *V. parahaemolyticus*- and WSSV-infected groups included many potentially involved in immunity, such as *caspase 4*, *integrin*, *toll receptor*, and *serine proteases*. Caspase-mediated apoptosis is important for anti-bacterial immunity in crustaceans [30,31], whild the apoptosis pathway is also involved in the immune responses of white-legged shrimp (*Litopenaeus vannamei*) to *V. parahaemolyticus* infection [32,33].

Integrins play crucial roles in cellular activities such as adhesion, migration, differentiation, proliferation, and apoptosis [34]. Various Toll components are activated in shrimps infected with *Vibrio* [35,36]. In crustaceans, melanin synthesis depends on the prophenoloxidase system and is involved in sclerotisation and wound healing of the cuticle, as well as defence reactions (nodule formation and/or encapsulation) against invading microorganisms [37,38]. Prophenoloxidase-activating proteins are the last proteases of the serine protease cascade that converts prophenoloxidase to phenoloxidase [39]. Among the 1,042 DEGs, immunity genes including *C-type lectins*, *$\alpha_2 M$*, and *heat shock proteins* were differentially expressed in the WSSV-infected group. C-type lectins participate in innate immunity and recognise and eliminate pathogens [40,41]. Expression of C-type lectins can be strongly affected following WSSV infection in the hepatopancreas of shrimps [42,43].

α_2M is a multifunctional player in the crustacean innate immune system [44,45]. As protease inhibitors, α_2M can neutralise pathogenic proteases that contribute to pathogen virulence and promote endocytic clearance [46]. Moreover, α_2M can crosslink with clotting proteins such as transglutaminase to form clots [47]. Heat shock proteins are well-known stress response factors involved in the responses to pathogenic infection in shrimps [48,49]. In the present study, we observed higher levels of expression of all heat shock proteins in *M. japonicus* when infected by *V. parahaemolyticus*. Increased expression of heat shock proteins is consistent with previous reports, which supports the important role of these proteins in protecting shrimps from the stress caused by *Vibrio* infection. These candidate genes provide an important basis for screening the key gene responses to *V. parahaemolyticus* and WSSV infection.

During infection by both *V. parahaemolyticus* and WSSV, 761 genes were significantly up- or down-regulated. Some known immunity-related genes, such as *thioredoxin*, *crustin*, and *ubiquitin-conjugating enzyme E2*, were included among them. Thioredoxin, a substrate for peroxiredoxin, is induced by oxidative stress resulting from viral infection [50], and a previous study demonstrated its possible role in the anti-*Vibrio* and anti-WSSV innate immunity of shrimps [51]. Crustins perform diverse biological functions that include protease inhibition and antimicrobial activities [52,53]. In the present study, crustin expression was affected by WSSV and *Vibrio* infection, similar to the findings of previous studies [53]. Ubiquitin-conjugating enzyme is an active component in the ubiquitin-mediated protein degradation pathway, and plays important roles in WSSV and *Vibrio* pathogenesis [54].

5. Conclusions

In this study, we investigated the transcriptome profiles of*M. japonicus* hepatopancreas following infection with *V. parahaemolyticus* or WSSV. We found evident changes following infection, with 1,466 host genes significantly up-regulated, and 1,573 genes significantly down-regulated. In WSSV-injected shrimps, 970 host genes were significantly up-regulated and 833 were significantly down-regulated. Additionally, 761 genes were differentially expressed in *V. parahaemolyticus* and WSSV-infected shrimps. Several genes known to be related to immune responses were identified among the differentially expressed transcripts. However, further studies on the functionality of these genes are required to enhance our understanding regarding the molecular interactions between *M. japonicus* and WSSV, which will likely provide valuable information for devising effective strategies to limit or prevent viral diseases.

Acknowledgments

This research was supported by the International Exchange and Cooperation in Agriculture, Ministry of Agriculture and Rural Affairs of China- Science, Technology and Innovation Cooperation in Aquaculture with Tropical Countries, and Shandong Leading Talent Program (No. LNJY2015002). We thank staff at the Key Laboratory for Sustainable Utilization of Marine Fisheries Resources for their help with sampling and shrimp rearing.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fsi.2019.02.030.

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