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Comparative transcriptomic analysis of crab hemocytes in response to white spot syndrome virus or *Vibrio alginolyticus* infection

Fei Zhu*, Xiyi Qian, Xiongchao Ma

College of Animal Science and Technology, Zhejiang Agriculture and Forestry University, Hangzhou, 311300, China

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ABSTRACT

To assess the immune response of hemocytes to white spot syndrome virus (WSSV) or *Vibrio alginolyticus* infection in the mud crab *Scylla paramamosain*, a transcriptome analysis was performed. We report the analysis of 45131 transcripts from *S. paramamosain* hemocytes by de novo assembly. A comparison with GenBank protein and nucleotide sequences identified 33699 genes as previously known. The length distribution of the genes was 8147 genes ≥200 bp, 4714 genes ≥300 bp, and 3517 genes ≥2000 bp. A total of 21579 simple sequence repeats (SSRs) were found in the transcriptomic dataset, including 9% monomers, 53.34% dimers and 32.55% trimers. A total of 13172 and 5087 differentially expressed transcripts were found in the *V. alginolyticus*-infected group and WSSV-infected group, respectively. Of these, 5920 transcripts were up-regulated and 7252 were down-regulated in the *V. alginolyticus*-infected crabs and 2302 transcripts were up-regulated and 2785 were down-regulated in the WSSV-infected crabs. Additionally, 3096 transcripts were differentially expressed simultaneously in the *V. alginolyticus*-infected crabs and the WSSV-infected crabs. Several known immune-related genes such as heat shock protein, Janus kinase, STAT, relish, caspase, Ca^{2+}-transporting ATPase and lysosomal alpha-mannosidase were found among the differentially expressed transcripts. Transcription and its regulation were significant biological processes, and ATP binding and zinc ion binding were significant molecular functions. This is the first report of comparative transcriptomic analysis of crab hemocytes in response to WSSV or *V. alginolyticus* infection. These findings will contribute to our understanding of the immune response to WSSV and *V. alginolyticus* infection in crustaceans.

1. Introduction

The mud crab *Scylla paramamosain* is widely distributed along the coastline of the southern China sea and has a high economic value. Little is known about the disease of *S. paramamosain* in the juvenile or adult phases, but susceptibility to infection has been identified during the larval stages. Several bacterial and viral pathogens have been reported to infect marine crabs and cause disease [1]. At present, bacterial infections are the main cause of crab disease and constraint in the crab farming industry [2,3]. In aquaculture, *Vibrio alginolyticus*, a species of Gram-negative bacteria, is an important bacterial pathogen which can cause huge economic losses and is responsible for many cases of natural infection in the Chinese mitten crab [4]. White spot syndrome has become the most hazardous and devastating disease in shrimp cultures worldwide [5], and it was found to infect some species of crab both in natural and experimental conditions [6–8].

Crustaceans, including crabs, only possess an innate immunity to defend against invading microbes [9]. The innate immune system in crustaceans includes phagocytosis, encapsulation, coagulation, melanization and antimicrobial peptides [9,10]. Crab hemocytes have been confirmed as the major target cells in white spot syndrome virus (WSSV) and *V. alginolyticus* infection [11,12]. The first transcriptome of the mud crab (*S. paramamosain*) was characterized by 454 deep sequencing and 78268 unigenes were identified based on sequence similarity with known proteins in UniProt and non-redundant protein databases [13]. The transcriptome analysis of the testis and ovary of *S. paramamosain* identified 4021 differentially expressed gonad genes, 10522 ovary-specific genes and 19013 testis-specific genes [14]. Some transcriptomic studies of disease resistance in *S. paramamosain* were also carried out. In a previous study, 4444 significantly up-regulated and 9412 down-regulated differentially expressed genes (DEGs) were detected in the gills of reovirus-infected mud crabs compared with the control [15]. In another study, 538 significantly up-regulated and 675 down-regulated genes were detected in hemocytes of *V. parahaemolyticus*-infected crabs compared to expression in the controls [16]. The hemocyte transcriptomic information for the crab response to

* Corresponding author.
E-mail address: zhufei@zju.edu.cn (F. Zhu).

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Table 1
General information of the transcriptome from *S. paramamosain*.

Dataset name	transcript	gene
All	45131	33699
Mean GC %	42.78	42.26
Min-Max length (bp)	201–20227	201–20227
Mean length (bp)	1177	904
N50 (bp)	2107	1488
Total Assembled bases	53154504	30475423

WSSV is still limited. Therefore, we analyzed the immune response to WSSV in hemocytes and compared the difference between WSSV and *V. alginolyticus* infection at the transcript level.

The objective of this work was to identify and annotate more immune-related genes in crab hemocytes and uncover the mechanisms of innate immune response to WSSV or *V. alginolyticus* challenges in crustaceans. In this study, we obtained 33699 genes and most of them were novel to *S. paramamosain*. Many genes were found to be related to the innate immune system in other species, such as pattern recognition receptors, antimicrobial peptides, proteases, signal transduction proteins, apoptosis-related proteins, and antioxidant proteins.

2. Materials and methods

2.1. Crab and tissue preparation

Healthy crabs *S. paramamosain* were kept in a 50 L tank containing sea water with an air pump at room temperature in our laboratory. WSSV (GenBank accession no. AF 332093.1) was prepared and used immediately for the challenge according to a previous report [17]. *V. alginolyticus* (ATCC17749) was cultured and used to challenge the crabs according to the method published in a previous report [18]. The hemocytes of pathogen-challenged crabs were collected for RNA isolation immediately and kept on ice to prevent RNA degradation. The hemocytes of crabs was aseptically collected from the nonsclerotized membrane of cheliped leg using 5 mL sterile syringe loaded with pre-chilled anticoagulant (20 mM EDTA) at the ratio of 1:1, then immediately centrifuged at 300 g at 4 °C for 10 min.

2.2. Pathogen challenge and mortality count

For the pathogen challenge, healthy crabs were randomly distributed into three groups (n = 10 per group) and each group has three biological replicates. All groups received injections in the third walking foot base of crabs. The control group received injections of PBS (phosphate buffer saline) alone, the WSSV/*V. alginolyticus* groups received injections of 100 μL WSSV (10^5 copies/mL)/*V. alginolyticus* (1.5×10^6/mL) in PBS. The mortality data was arranged and analyzed in Microsoft Excel (2003). The mortality of WSSV-challenged crabs reached ~70% and that of *V. alginolyticus* group reached ~90% at 7 days post challenge. The control group showed no mortality at 7 days post challenge. The samples were collected at 24 h post challenge and RNA isolation was performed for Illumina sequencing and real-time quantitative PCR.

2.3. RNA isolation and illumina sequencing

Total RNAs were isolated from the lymphoid organs of the infected and non-infected crab at different times after infection using Unizol reagent (UnionGene, China) and treated with DNase I, and the mRNAs were purified from the total RNAs using the PolyA Ttract mRNA isolation systems (Promega, USA) following the manufacturer's instructions. The quantity and purity of total RNAs were monitored using a NanoDrop ND-1000 spectrophotometer (Nano Drop, DE). The RNA sequencing was performed by Lianchuan Biotechnology Co. Ltd. (China) on an Illumina Hiseq2000/2500 system according to the manufacturer's instructions.

2.4. De novo assembly and gene annotation

Raw sequencing reads were quality trimmed, and adaptor sequences were removed before the assembly. The processed reads were assembled using Trinity software with default parameters (ref: http://www.ncbi.nlm.nih.gov/pubmed/21572440). The derived gene sequences were searched against NCBI non-redundant protein sequence database for annotation, with e-value cutoff being 1×10^{-10}. For Gene ontology (GO) annotation, InterproScan was used for obtaining GO term (ref: http://nar.oxfordjournals.org/content/43/D1/D213), and WEGO (Web Gene Ontology) was used for plotting GO annotation results [19]. Determination of eukaryotic complete genomes (KOGs) type followed the method as described (ref: http://www.ncbi.nlm.nih.gov/pubmed/14759257). The annotation of KEGG (Kyoto Encyclopedia of Genes and Genomes) orthology and pathway was done by using the KEGG Automatic Annotation Server (ref: http://nar.oxfordjournals.org/cgi/content/full/35/suppl2/W182). Estimation of expression abundances and identification of differentially expressed genes were done by using Cuffdiff program in the software package Cufflinks with default parameters (http://cole-trapnell-lab.github.io/cufflinks/).

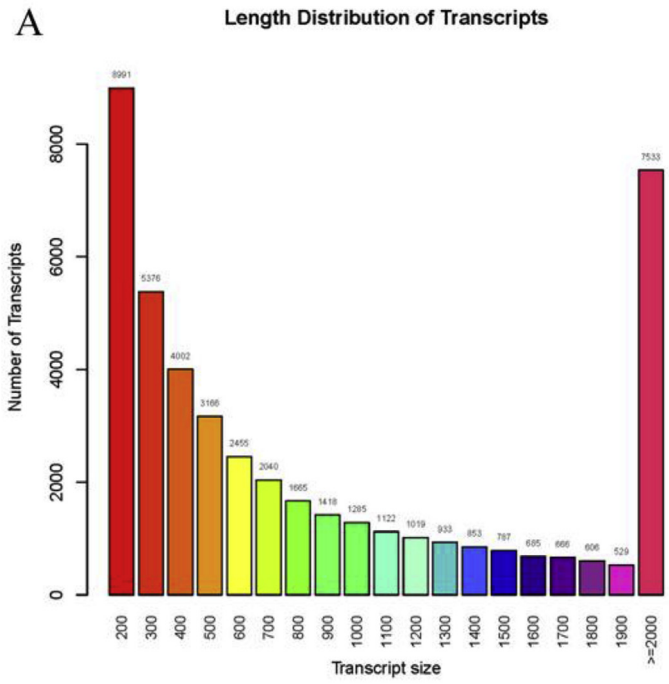
2.5. Expression analysis by real-time PCR

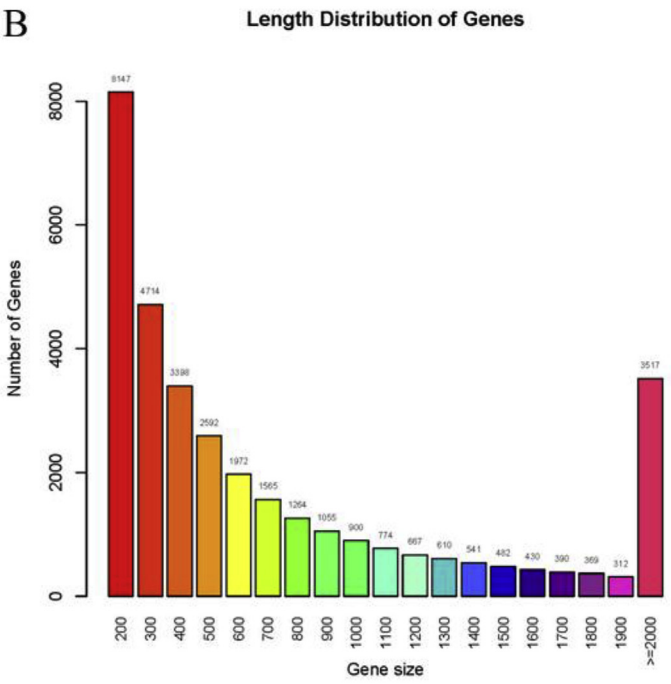
The expression levels of a gene in multiple organs of both healthy and pathogen-challenged crabs were analyzed by real-time quantitative PCR using SYBR Green qRT-PCR assay (Promega, USA). The procedure was done according to the published method described previously [20]. And the primers were shown in Table S1. These genes were chosen for their known highly homologous genes in other animals and their high fold change in gene expression.

3. Results

3.1. Sequencing and de novo assembly

We report the analysis of 45131 transcripts (mean length = 1177 bp) from *S. paramamosain* hemocytes by de novo assembly. Comparison with GenBank protein and nucleotide sequences identified 33699 genes as previously known genes with an average length of 904 bp (Table 1). The length distribution of the transcripts were 8991 genes ≥200 bp, 5376 genes ≥300 bp, 4002 genes ≥400 bp, and 7533 genes ≥2000 bp (Fig. 1A). The length distribution of the genes were 8147 genes ≥200 bp, 4714 genes ≥300 bp, 3398 genes ≥400 bp, and 3517 genes ≥2000 bp (Fig. 1B). The species distribution of the BLASTX results showed the





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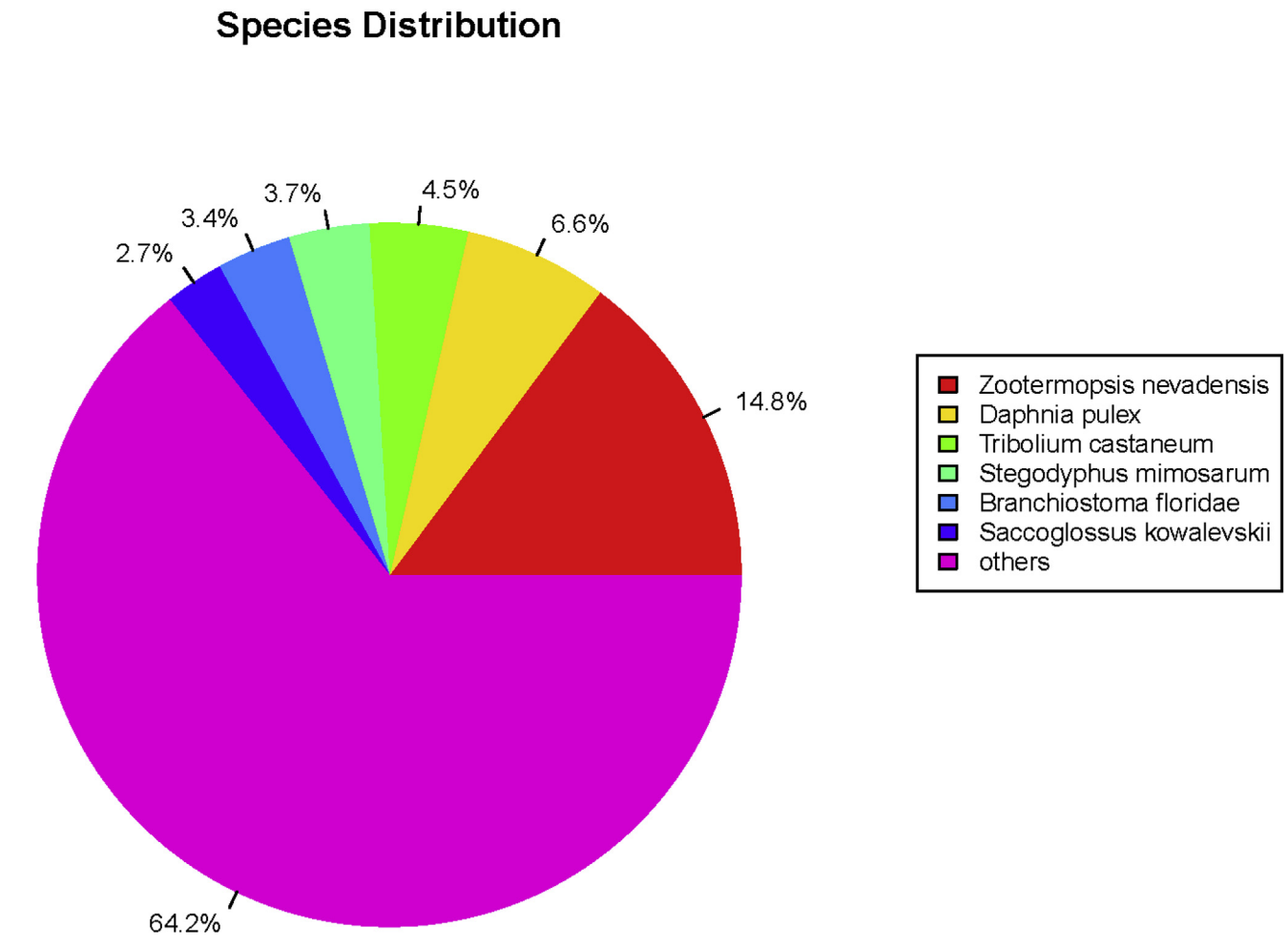


(caption on next page)

Fig. 1. (A) Lenghth distribution of *S. paramamosain* transcripts. (B) Lenghth distribution of genes. (C) Species distribution of the BLASTX results. The figure shows the species distribution of gene BLASTX results against the NCBI non-redundant protein databases with a cutoff E-value < 10^{-5}. Different colors represent different species. Species with proportions of more than 1% are shown. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

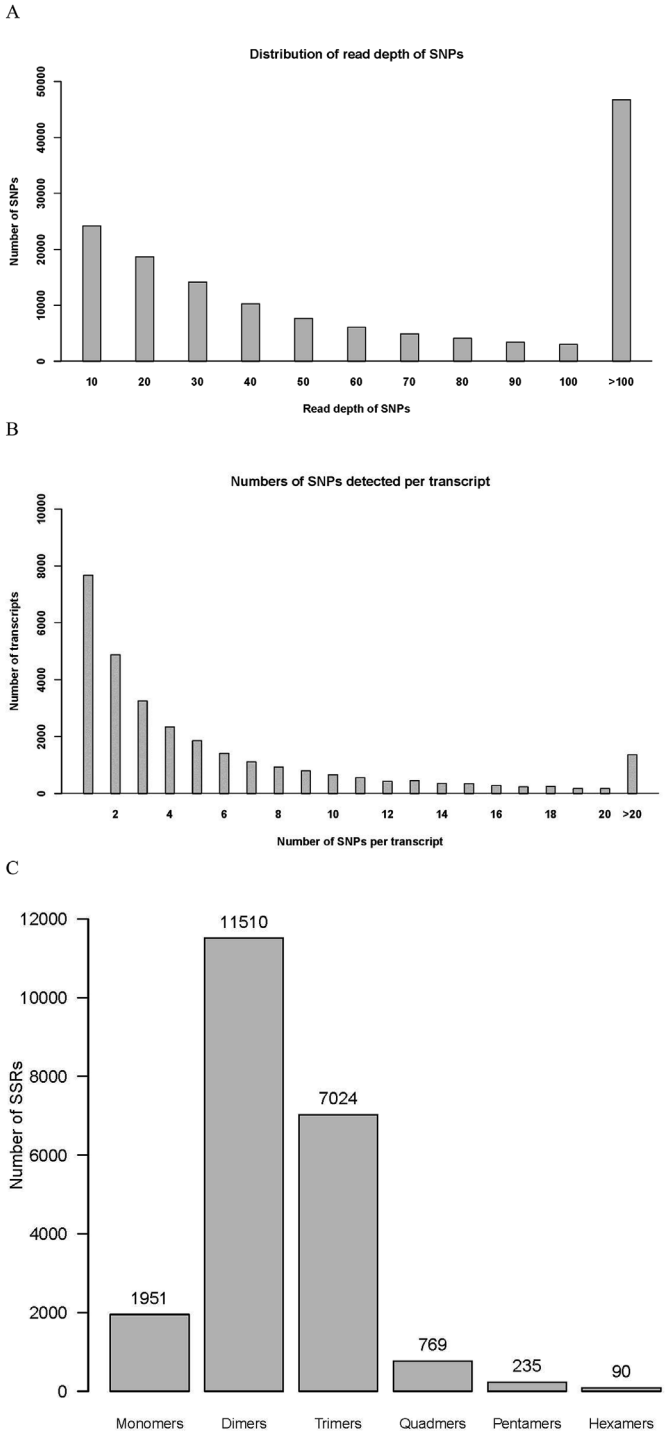


Fig. 2. (A) Distribution of read depth of SNPs in the transcriptome of *S. paramamosain*. (B) Numbers of SNPs detected per transcript. (C) Distribution of simple sequence repeat (SSR) nucleotide classes among different nucleotide types found in the transcriptome of *S. paramamosain*.

species proportion of *Zootermopsis nevadensis* (14.8%), *Daphnia pulex* (6.6%), *Tribolium castaneum* (4.5%), *Stegodyphus mimosarum* (3.7%), *Branchiostoma floridae* (3.4%), *Saccoglossus kowalevskii* (2.7%) and others (64.2%) (Fig. 1C).

3.2. Similarity searches

A total of 19910 *S. paramamosain* coding DNA sequences (CDS) were used for the sequence similarity searches and BLASTx results were obtained. All of the CDS with BLASTx results were used for the species distribution analysis. The species distribution of the best-matched result for each sequence is shown in Fig. 1C. Overall, 2947 (14.8%), 1314 (6.6%) and 896 (4.5%) *S. paramamosain* CDS showed strong similarity to *Zootermopsis nevadensis*, *Daphnia pulex*, and *Tribolium castaneum*, but 12782 (64.2%) shared a high homology with other species.

3.3. SNP and SSR discovery

Single-nucleotide polymorphisms (SNPs) are the most common type of variation in the genome. The number of > 100 bp SNPs is over 4000 in the transcriptome of *S. paramamosain*, > 10 bp SNPs is over 2000 and the other length SNPs is under 2000 (Fig. 2A). The number of most SNPs detected per transcript is one and the number decreased gradually with the increase of SNPs (Fig. 2B). SSRs are short sequences of one to five nucleotides that are repeated several to many times in eukaryotic genomes. A total of 21579 simple sequence repeats (SSRs) were obtained from the transcriptomic dataset, including 9% monomers, 53.34% dimers and 32.55% trimers (Fig. 2C).

3.4. GO and KEGG analysis of differentially expressed genes (9374)

The transcripts were aligned to Gene Ontology (GO) terms (Fig. 3), 10837 transcripts were mainly divided into three categories with 59 functional groups: biological process (25 functional groups), cellular component (15 functional groups), and molecular function (10 functional groups). Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway classification was performed to analyze DEGs (9374). The KEGG pathway analysis indicated that more transcripts (793 transcripts) were classified in the signal transduction pathway than in any other pathway (Fig. 4). Metabolism was a very significant pathway within the larger category. A total of 12339 transcripts were used for the KOG functional categories (Fig. 5). The data showed that general function prediction and signal transduction mechanisms are very significant functions.

3.5. Transcriptome comparison between WSSV/V. alginolyticus infected and uninfected crab

The crabs were challenged with *V. alginolyticus* or WSSV. Samples were then used for transcriptome analysis. WSSV and *V. alginolyticus* infection resulted in 35340554 and 30569310 raw reads respectively (Table 2). After removal of repetitive, low-complexity, and low-quality reads, 34770360 and 30247918 clean reads were harvested for the

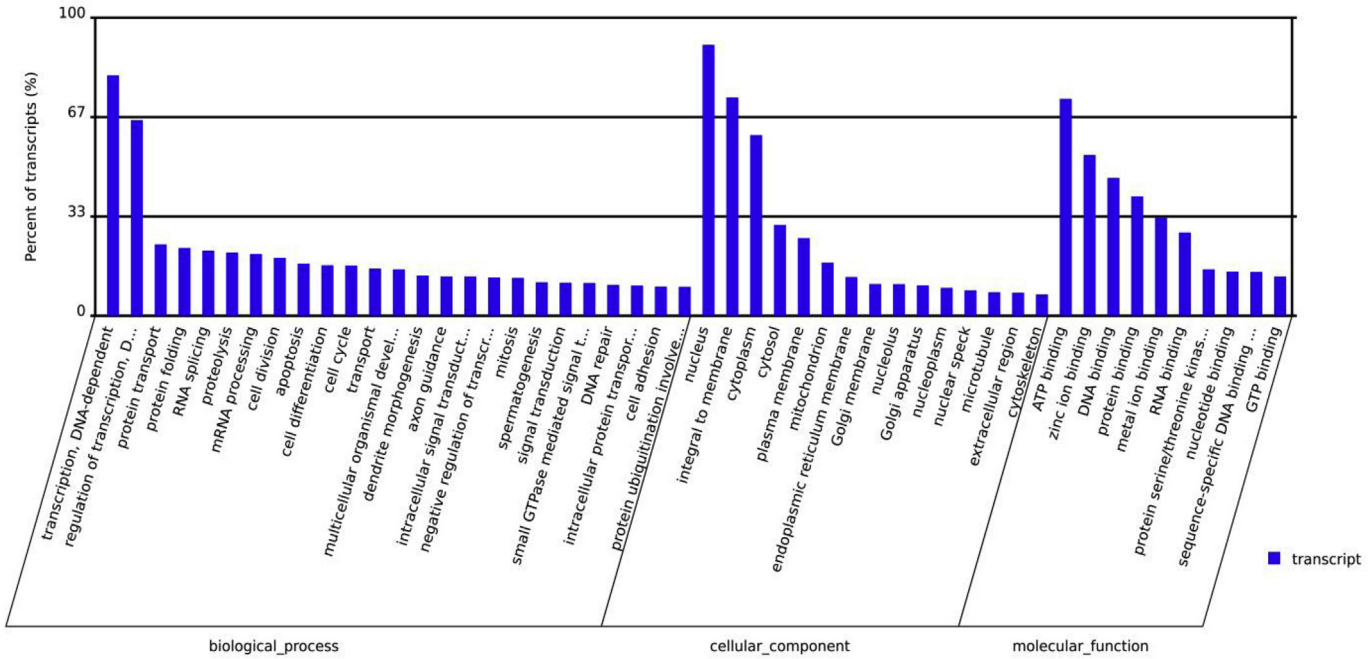


Fig. 3. Gene ontology classification of hemocytes transcripts in health *S. paramamosain*. The left y-axis indicates the percentage of a specific category of transcripts existed in the main category.

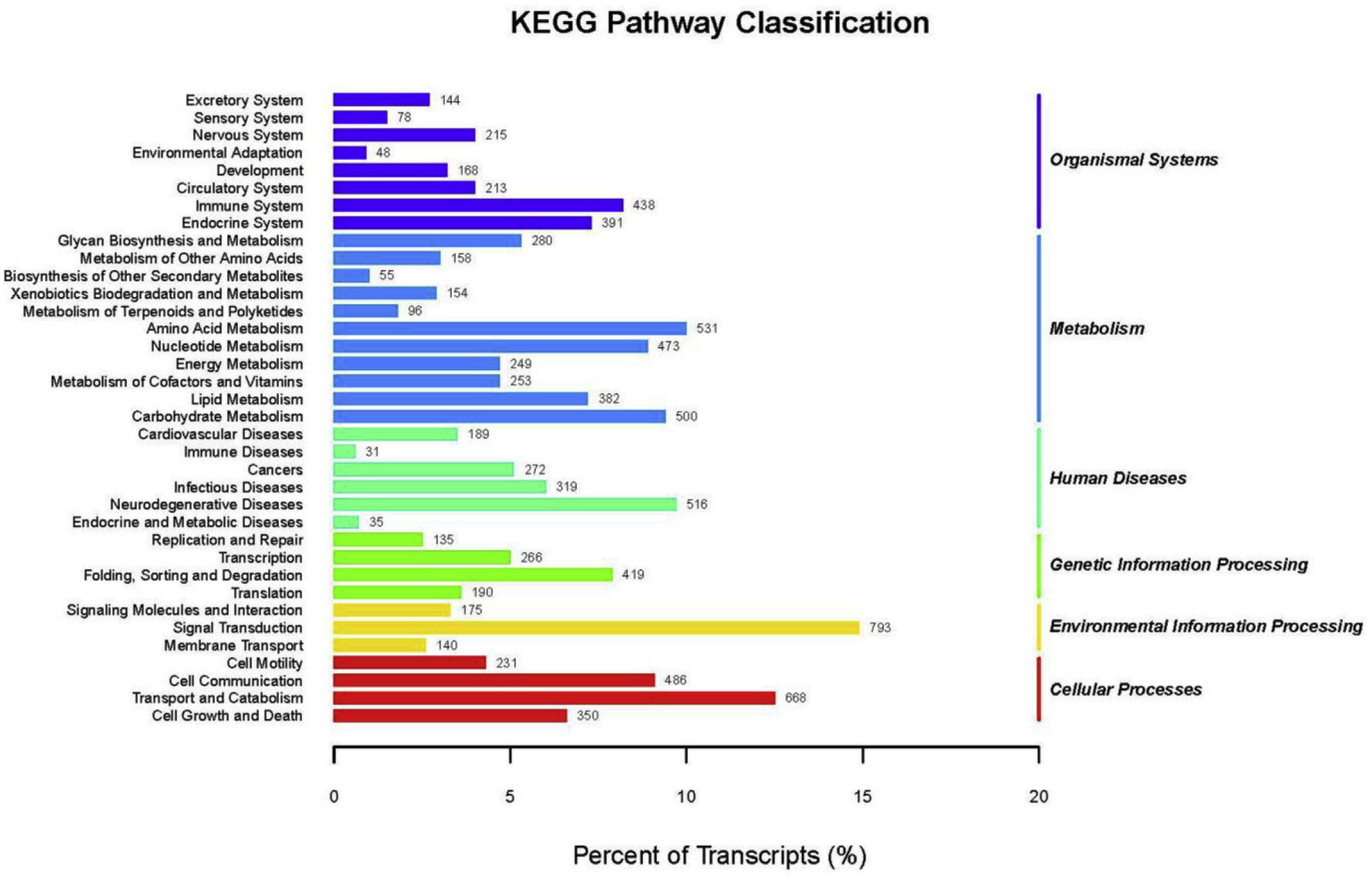


Fig. 4. Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway classification of hemocytes transcripts in health *S. paramamosain*.

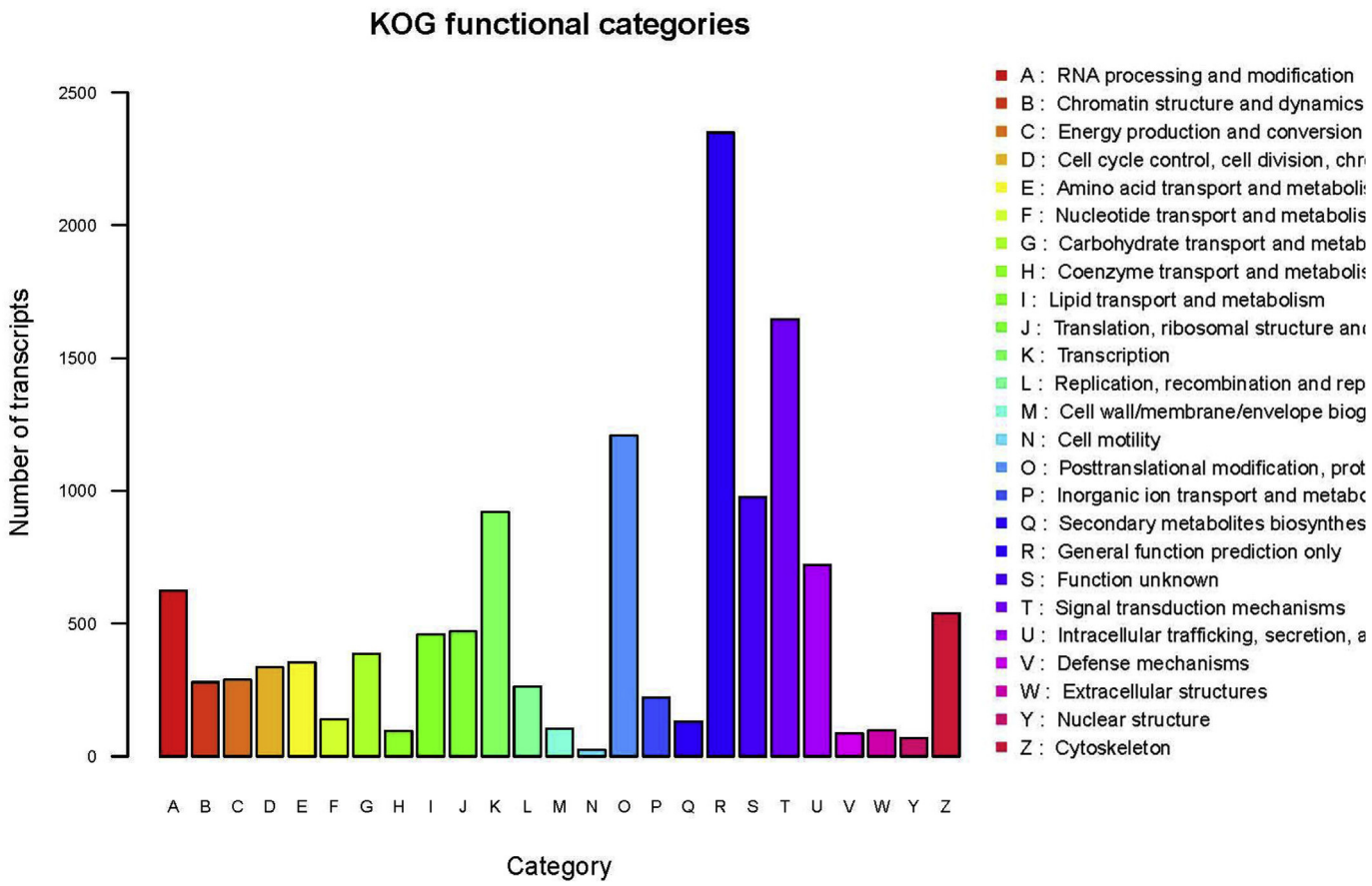


Fig. 5. Eukaryotic cluster of orthologous groups (KOG) function classification of hemocytes transcriptome in health *S. paramamosain*.

Table 2
General information of the transcriptome from challenged *S. paramamosain*.

Dataset name	Control	WSSV	*V. alginolyticus*
Total raw reads (paired-end)	39201426	35340554	30569310
Total clean reads	38753952	34770360	30247918
Valid %	98.86%	98.39%	98.95%
Q20 percentage	87.69%	91.48%	88.50%
Q30 percentage	77.67%	83.94%	78.67%
GC percentage	45.75%	44.17%	45.05%

transcriptome analysis of WSSV and *V. alginolyticus* infection (Table 2). A total of 39201426 raw reads and 38753952 clean reads were obtained from the control (Table 2). We applied a 2-fold ($P < 0.05$) increase or decrease as a threshold for significant differences in transcription to qualify a physiologically important change. *V. alginolyticus* infection causes more gene expression to be up-regulated or down-regulated than WSSV infection. *V. alginolyticus* infection causes 13172 transcripts to be up-regulated or down-regulated above 2-fold ($P < 0.05$), including 5920 up-regulated transcripts and 7252 down-regulated transcripts (Fig. 6A). And WSSV infection causes 5087 transcripts to be up-regulated or down-regulated above 2-fold ($P < 0.05$), including 2302 up-regulated transcripts and 2785 down-regulated transcripts (Fig. 6A). Interestingly, among these transcripts, 3096 transcripts were up-regulated or down-regulated significantly in both WSSV and *V. alginolyticus* infection (Fig. 6B).

3.6. GO and KEGG analysis of 3096 transcripts

We used 3096 differentially expressed transcripts in GO classification (Fig. 6C). In the biological process, the significant processes are transcription and regulation of transcription. In the cellular component

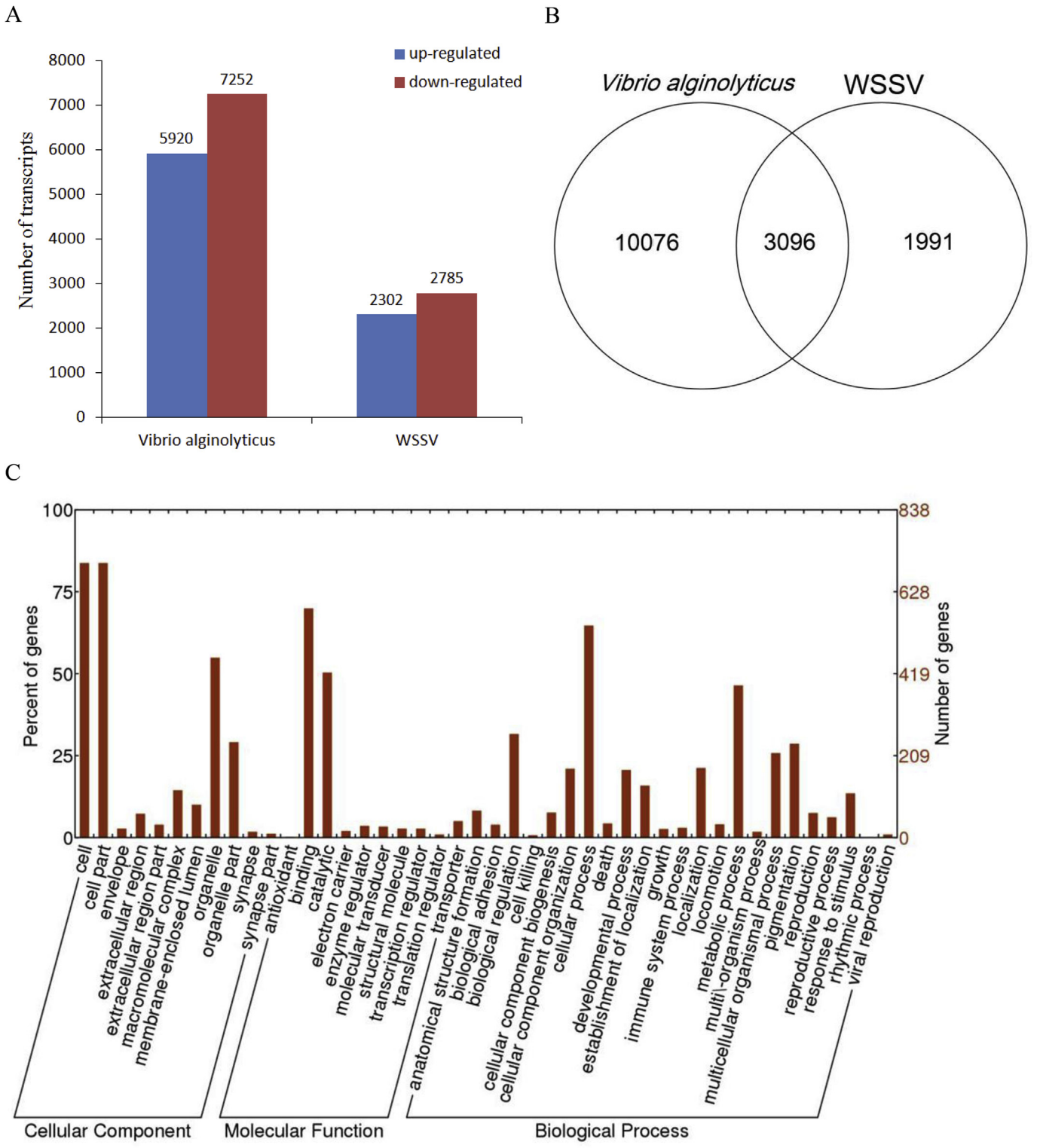


Fig. 6. The differentially expressed transcripts were evaluated by bioinformatics analysis. The numbers represent the transcripts which were up-regulated or down-regulated (more than 2 fold) compared with the control group (A). (B) Venn diagram of differentially expressed transcripts. The numbers represented the transcripts up-regulated or down-regulated (more than 2 fold) compared with the control. The Gene ontology classification (C) of the 3096 differentially expressed transcripts in *V. alginolyticus* group and WSSV group. (D) KEGG pathway classification.

D

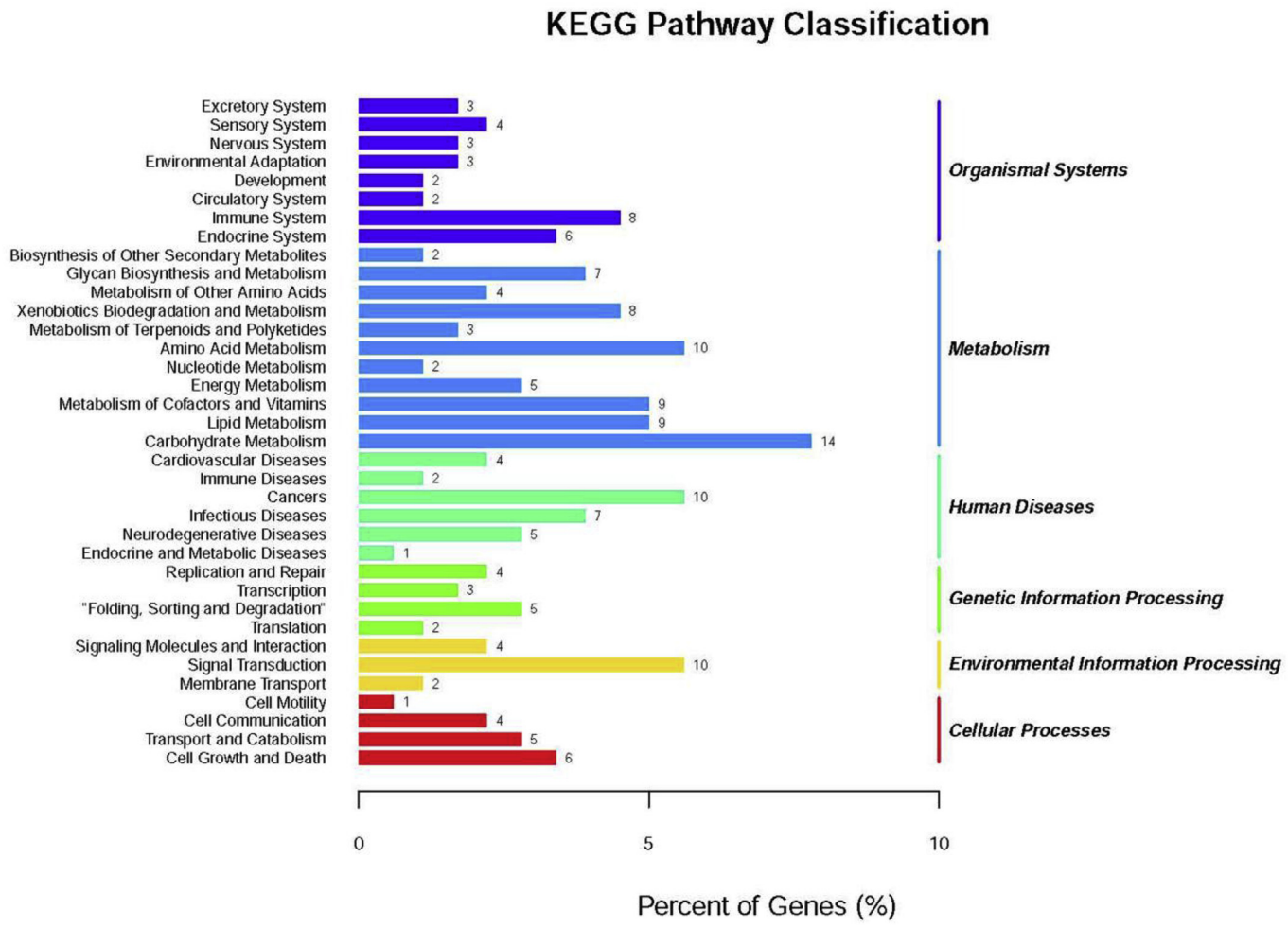
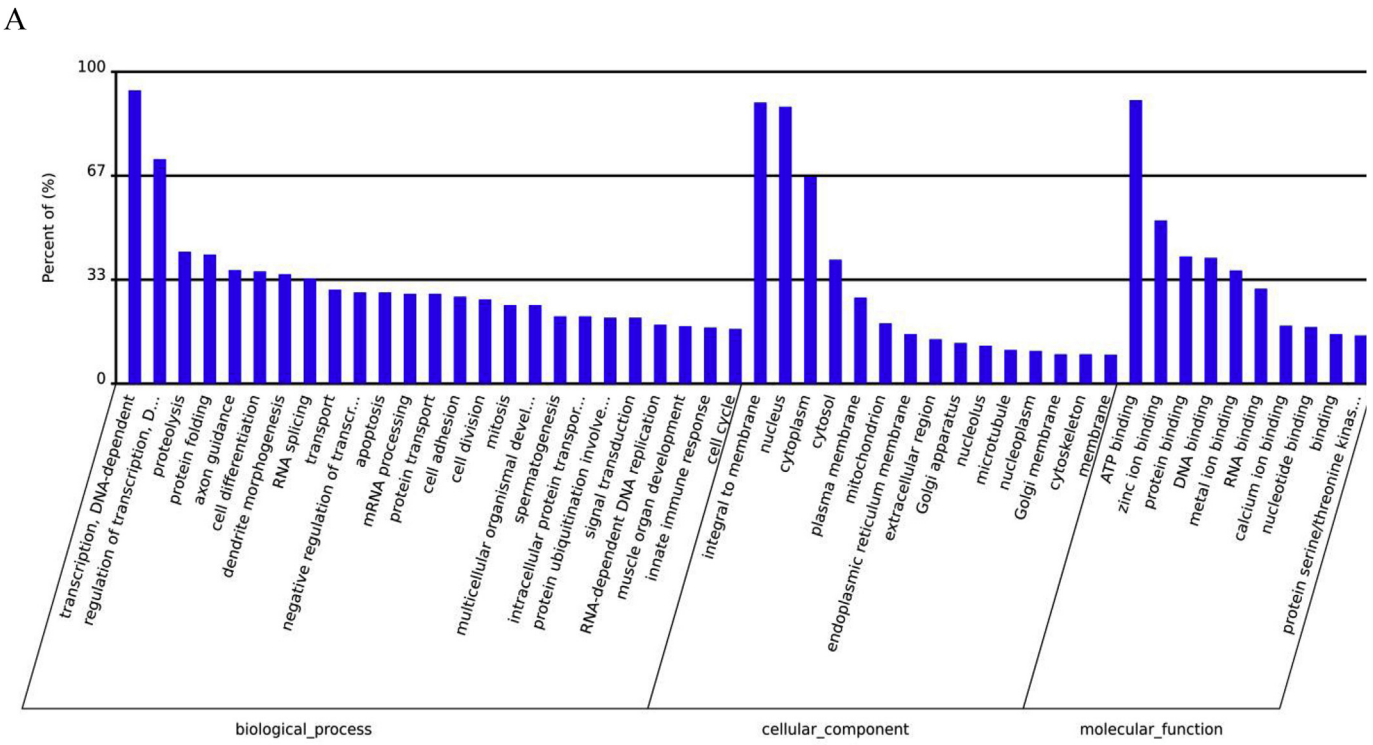


Fig. 6. (*continued*)

classification, transcripts were overrepresented in the nucleus, integral membrane and cytoplasm categories. In molecular function, they were overrepresented in ATP binding and zinc ion binding. Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway classification was performed to analyze the 3096 DEGs (Fig. 6D). Heatmap cluster analysis was performed with the normal and challenged hemocytes transcriptome in *S. paramamosain* (Fig. S1). The results showed that the *V. alginolyticus* transcriptome had a greater similarity to the WSSV transcriptome than the control.

The GO classification of the *V. alginolyticus* transcriptome showed that transcription is the most represented biological process, integral to membrane and nucleus are significant in cellular component, and ATP binding is overrepresented in molecular function (Fig. 7A). Calcium ion binding is the most enriched in the GO analysis (Fig. 7B). Calcium signaling pathway and phagosome are significant in the statistics of pathway enrichment (Fig. 7C). KEGG statistics showed that 8% down-regulated transcripts contribute to purine metabolism and 7% up-regulated transcripts contribute to endocytosis (Fig. 7D). The GO classification of the WSSV transcriptome showed that transcription is the most important biological process, integral to membrane and nucleus are significant in cellular component, and ATP binding is the most significant molecular function (Fig. 8A). The cytoplasm is the most



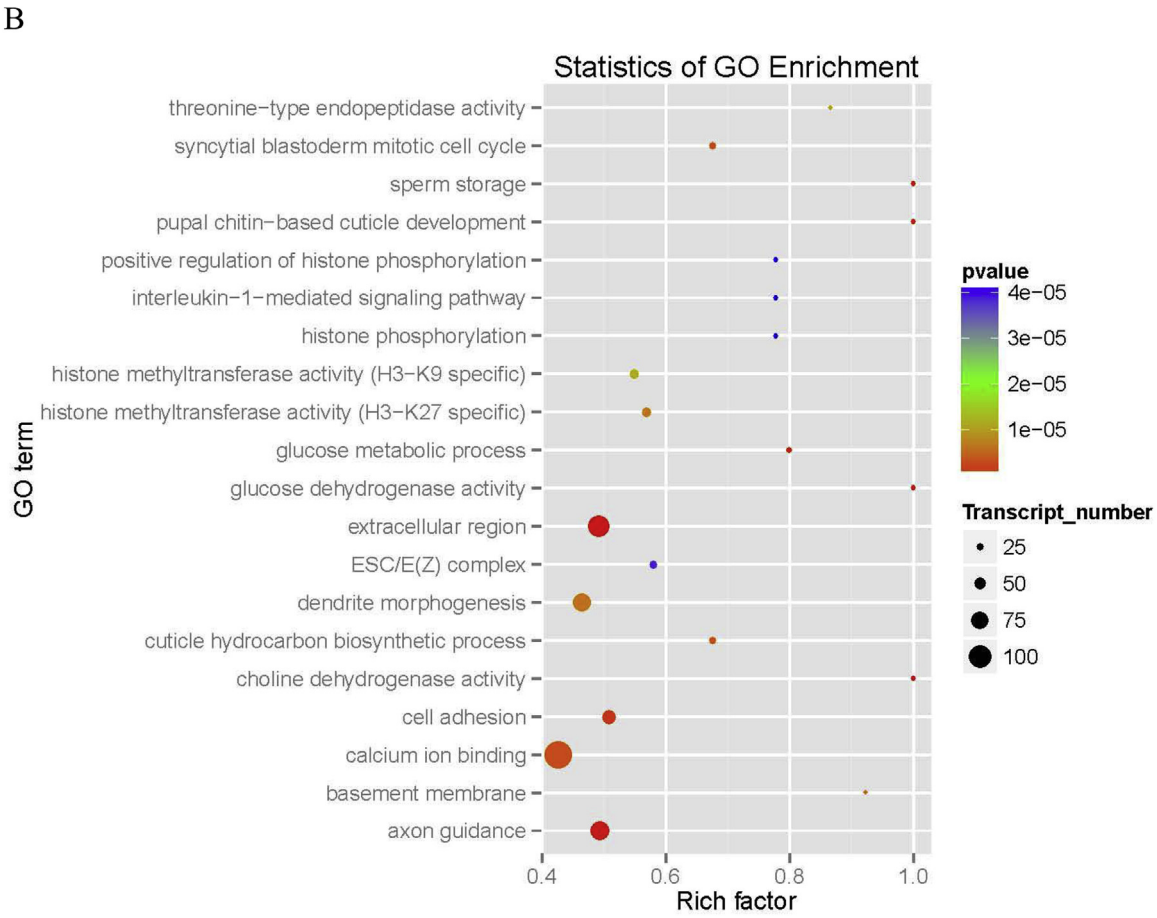
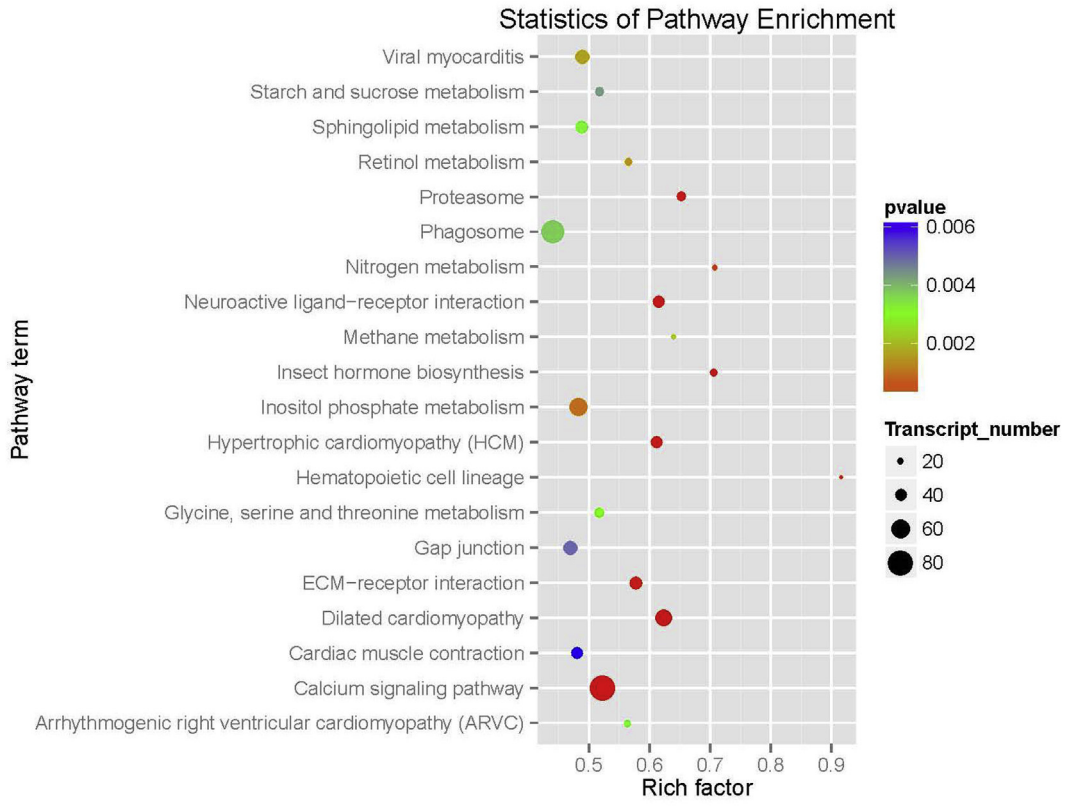


Fig. 7. The gene ontology classification (A), statistics of GO enrichment (B), statistics of pathway enrichment (C) of differential expressed transcripts (more than 2 fold, 13172) from *V. alginolyticus* group vs control group. (D) KEGG statistics of these up-regulated or down-regulated transcripts in abundance.

C



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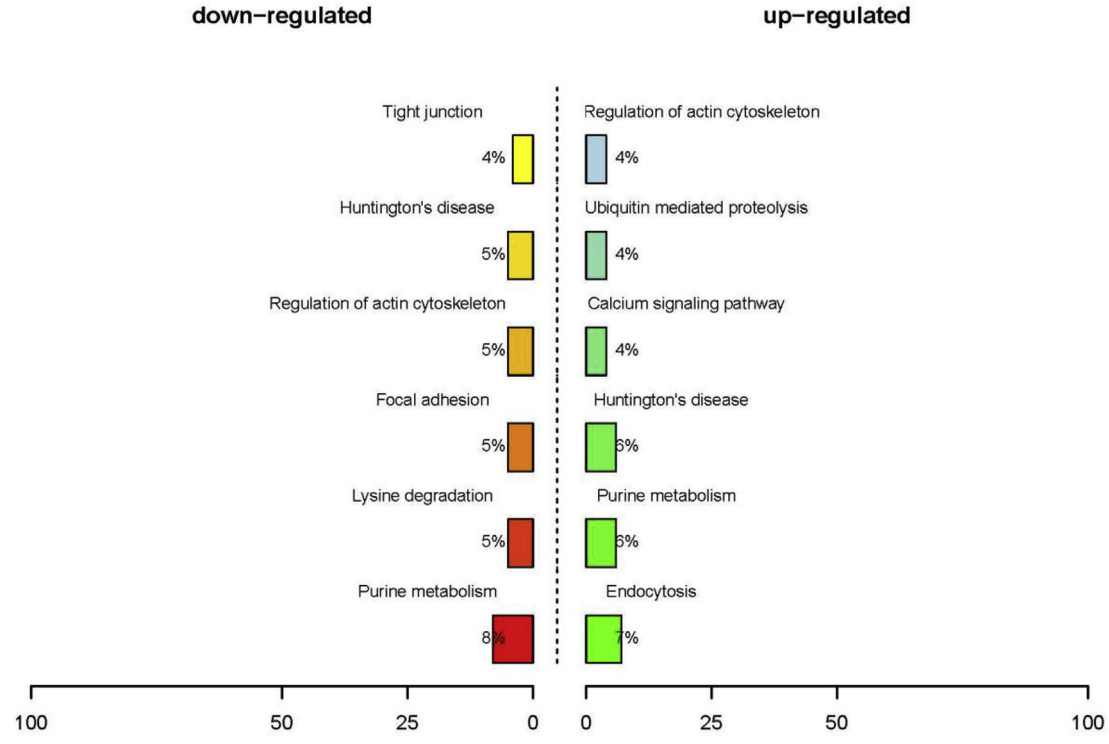
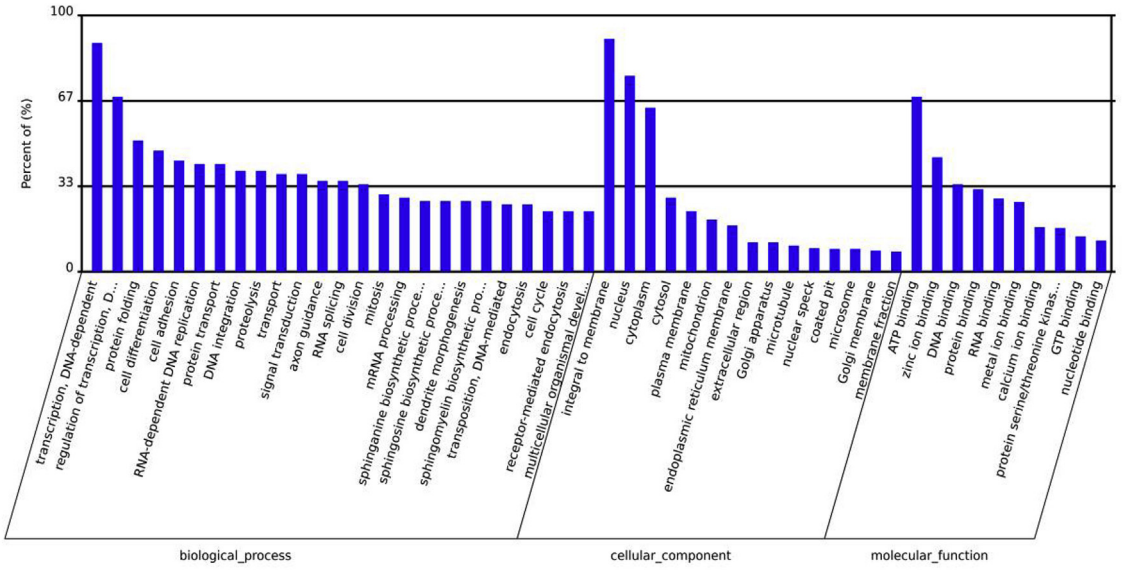


Fig. 7. (*continued*)

A



B

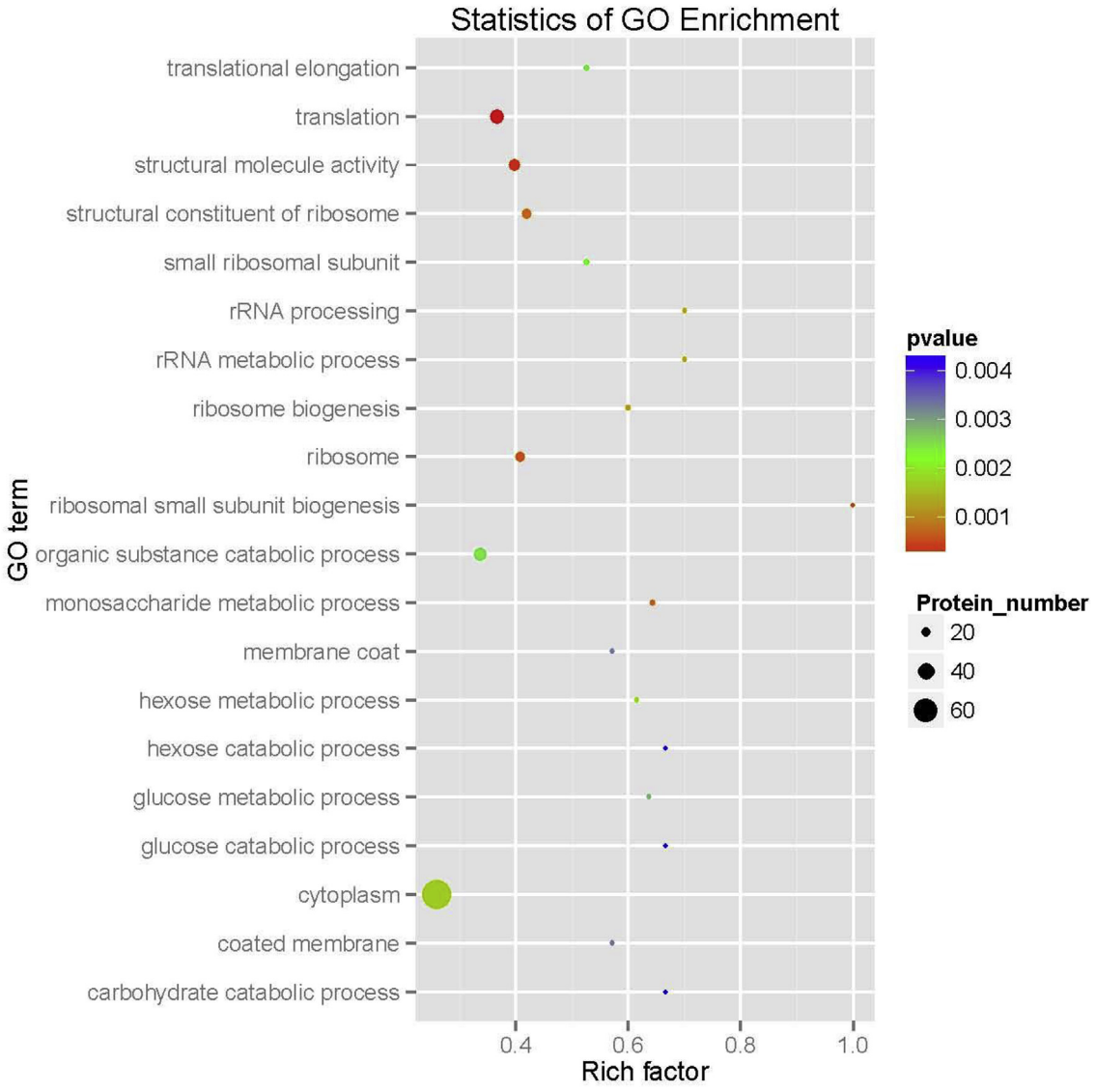


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Fig. 8. The gene ontology classification (A), statistics of GO enrichment (B), statistics of pathway enrichment (C) of differential expressed transcripts (more than 2 fold, 5087) from WSSV group vs control group. (D) KEGG statistics of these up-regulated or down-regulated transcripts in abundance.

significant in the statistics of GO enrichment (Fig. 8B). Lysine degradation pathway and phagosome are significant in the statistics of pathway enrichment (Fig. 8C). KEGG statistics showed that 9% of down-regulated transcripts contribute to the lysine degradation pathway and 13% of up-regulated transcripts contribute to purine metabolism, which also possesses 8% of down-regulated transcripts (Fig. 8D).

3.7. Identification of differentially expressed genes

As the 3096 DEGs were up-regulated or down-regulated significantly in both WSSV and *V. alginolyticus* infection, some candidate genes were chosen for further investigation (Table 3). Most of the genes were up-regulated or down-regulated simultaneously in the *V. alginolyticus* and WSSV transcriptome, including some known immune-related genes like heat shock protein, Janus kinase, STAT, relish, caspase, Ca^{2+}-transporting ATPase, and lysosomal alpha-mannosidase. Six DEGs, *Glutaminyl-peptide cyclotransferase*, *Troponin T*, *Janus kinase*, *Matrix metalloproteinase*, *Relish* and *Carboxypeptidase A* were characterized by real-time PCR assay (Fig. 9). The expression of these six genes showed a similar trend to the transcriptome results. Only *Glutaminyl-peptide cyclotransferase*, *Troponin T*, and *Carboxypeptidase A* were significantly ($P < 0.01$) up-regulated or down-regulated simultaneously in *V. alginolyticus* and WSSV infection.

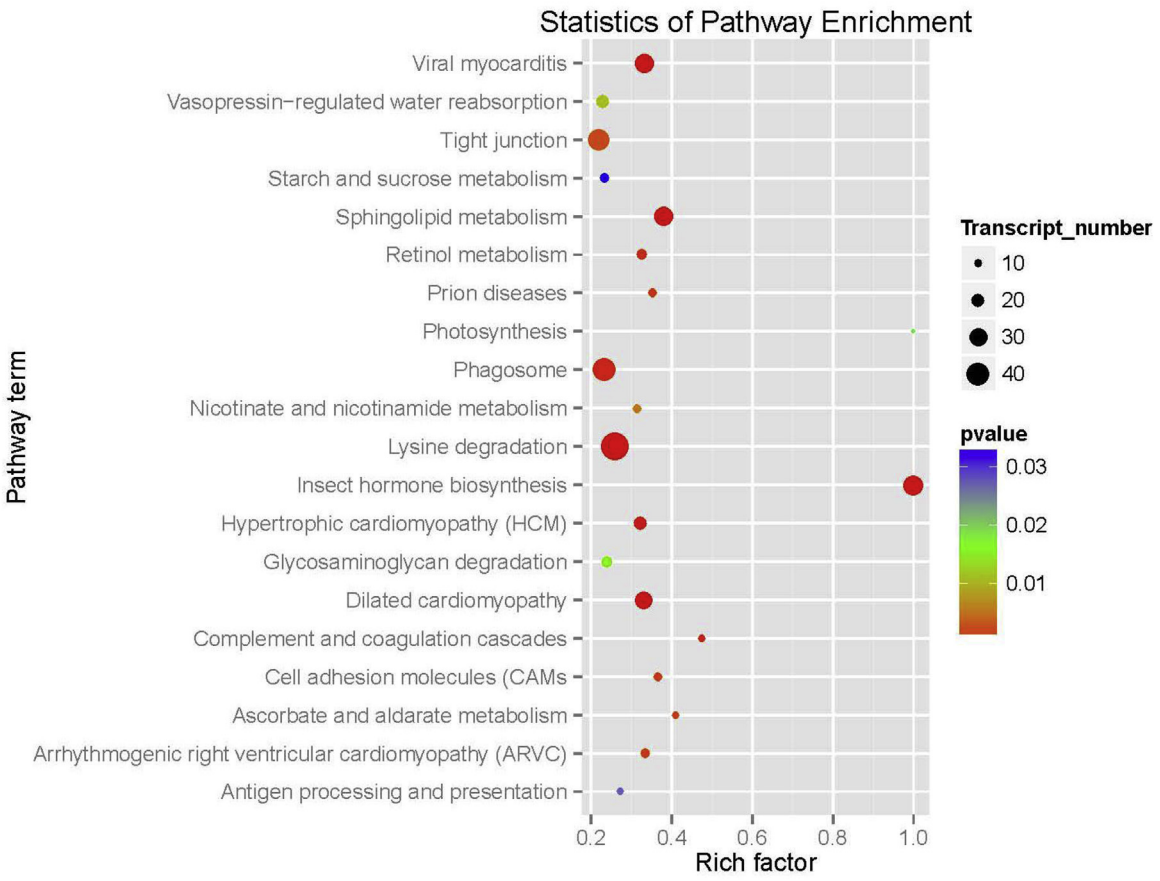
4. Discussion

The first transcriptome analysis of muscle tissue from *S. paramamosain* by 454 deep sequencing identified 78268 unigenes with a mean length of 420 bp [13]. In this study, we reported 45131 transcripts from *S. paramamosain* hemocytes by de novo assembly, and 33699 genes were identified as known genes with an average length of 904 bp. SNPs provide the best genome coverage for analyzing the performance and production of traits. The number of > 100 bp SNPs is over 4000 in the transcriptome of *S. paramamosain*, > 10 bp SNPs is over 2000 and the other length SNPs is under 2000. A total of 37,063 potential SNPs were identified from muscle tissue of *S. paramamosain* and the overall density of SNP was one every 466 bp [13]. The number of most SNPs detected per transcript is one and the number decreased gradually with the increase of SNPs. SSRs have become useful markers in aquatic organism genetic research due to their abundance in the genome, co-dominant nature, high polymorphism and ability to reproduce [21]. In this study, 21579 SSRs were obtained in the transcriptomic dataset, including 9% monomers, 53.34% dimers and 32.55% trimers. The BLASTx results revealed that 14.8% of the *S. paramamosain* coding sequence showed strong similarity to *Z. nevadensis*. But 8610 SSRs obtained from the ovary and testis transcriptome and the *S. paramamosain* coding sequence showed strong similarity to the water flea *Daphnia pulex* [14]. GO classification of hemocyte transcripts showed that transcription and regulation of transcription are significant biological processes; nucleus, integral membrane and cytoplasm are significant cellular components; ATP binding and zinc ion binding are significant molecular functions. In the molecular function category of the ovary and testis transcriptome from *S. paramamosain*, catalytic activity (44.69%) and binding (43.29%) were the most abundant [14]. Signal transduction pathways and metabolism were identified as significant in KEGG pathway analysis of our results. In other studies, metabolic pathway was the most statistically significant categories in KEGG classification of *S. paramamosain* transcriptome [14,16].

V. alginolyticus is a major pathogenic bacterium in marine crabs farming. *V. alginolyticus* infection can damage the mitochondria of hemocytes and then cause the death of hemocytes in crabs [22]. In this study, we investigated the transcriptome profile of *V. alginolyticus*-infected and WSSV-infected *S. paramamosain* and carried out a comparative analysis. Interestingly, we found that *V. alginolyticus* infection significantly affected the expression of more transcripts in host hemocytes than WSSV infection. In previous studies, 13856 DEGs were detected in the gills of reovirus-infected mud crabs compared with the control [15], but only 1213 DEGs were detected in hemocytes of *V. parahaemolyticus*-infected crabs compared with the control [16]. In both WSSV infection and *V. alginolyticus* infection, transcription is the most significant biological process, integral to membrane and nucleus are significant in cellular component, ATP binding and purine metabolism are significant molecular functions. The difference lies in that calcium signaling pathway and endocytosis are significant for *V. alginolyticus* infection, but lysine degradation pathway and phagosome are significant for WSSV infection. Similarly, regulation of actin cytoskeleton was significant for *S. paramamosain* during *Vibrio parahaemolyticus* infection [16]. In this study, 3096 transcripts were up-regulated or down-regulated significantly in both WSSV and *V. alginolyticus* infection; some known immune-related genes such as Janus kinase, STAT, heat shock protein, relish, caspase, Ca^{2+}-transporting ATPase, and lysosomal alpha-mannosidase were found among them. Janus kinase and STAT are key members of the JAK/STAT signaling pathway [23,24], which plays an important role in the innate immunity of crustaceans especially in antiviral and antibacterial immune responses [25–28]. The heat shock proteins are well-known stress response proteins and are involved in the response to pathogenic challenges in crabs [29–31]. Relish, a novel NF-κB-like transcription factor, essentially regulates immune-related gene expression and participates in antibacterial defense in crabs [32,33]. The caspase-mediated apoptosis is very important for the antiviral immunity of crustaceans [34,35], and apoptosis pathway was involved in *S. paramamosain* against mud crab reovirus [15]. Ca^{2+}-transporting ATPase, which activates the calcium signal transduction pathway, is involved in the response to stress, such as low salinity, and induces apoptosis in crabs [36,37]. Our study confirmed that these proteins all participate in the immune response to both WSSV infection and *V. alginolyticus* infection.

This is the first reported transcriptome analysis of immune-related genes from crustacean hemocytes in response to WSSV and *V. alginolyticus* infection. The findings will contribute to our understanding of the molecular mechanisms of immune response to WSSV or *V. alginolyticus* infection in crustacean.

C



D

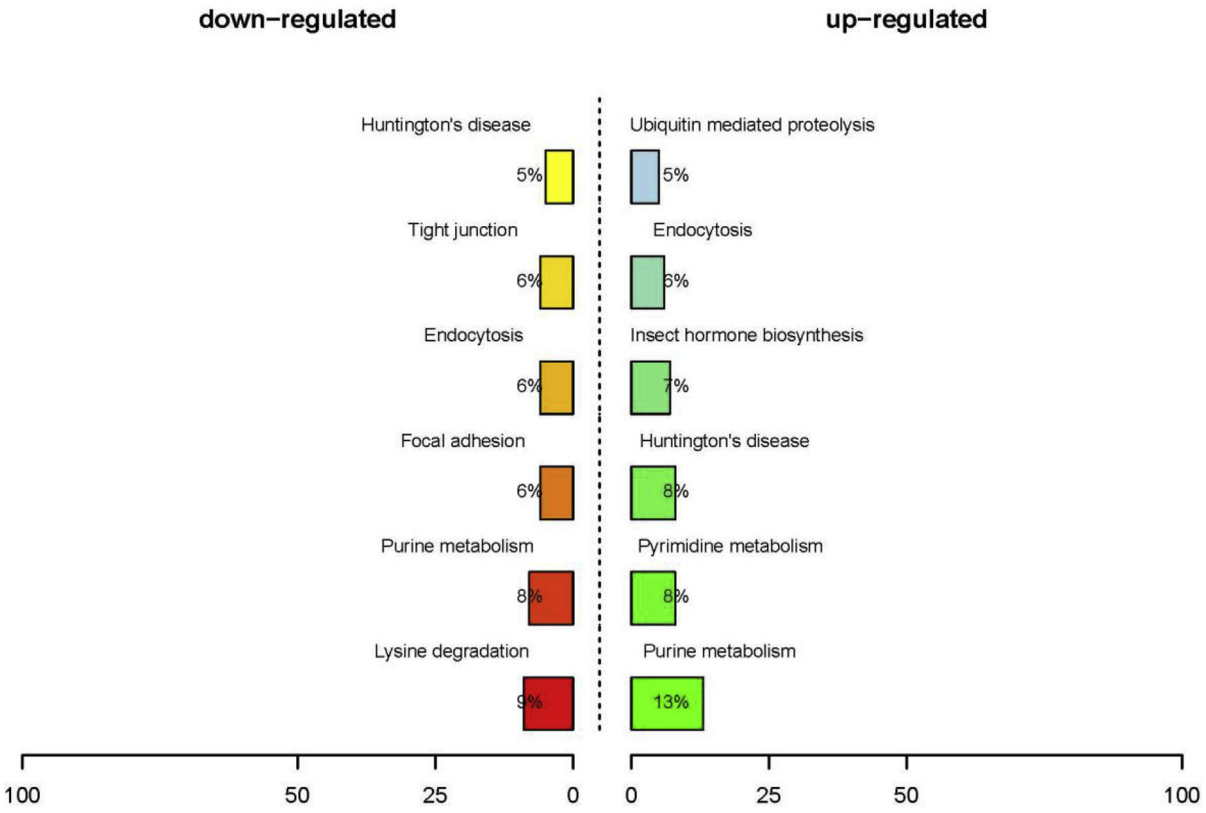


Fig. 8. (*continued*)

Table 3
Candidate genes involved in the immune response of *S. paramamosain*.

Gene ID	WSSV FC	VA FC	Annotation	Length (kb)
comp10311_c0	2.29	16.41	heat shock protein 90 kDa beta	2999
comp10516_c0	0.086	0.003	troponin T	1182
comp10861_c1	2	2	Ca^{2+}-transporting ATPase	396
comp10926_c0	0.32	0.11	transcription factor SOX4	310
comp11138_c1	2	179	suppressor of tumorigenicity protein	860
comp11180_c0	8.65	39.46	casein kinase 1, epsilon	1817
comp114140_c0	0.35	0.04	collagen, type I/II/III/V/XI, alpha	634
comp11448_c2	0.088	0	myosin-light-chain kinase	1762
comp11858_c0	0.38	0.08	cell division cycle 2-like	2628
comp11924_c0	0.32	0.17	Janus kinase 2	857
comp12223_c0	4.63	181.87	Prss55, trypsin	1375
comp12583_c0	0.08	0.008	CYIIA, actin beta/gamma 1	313
comp12697_c0	13.80	122.38	endoglucanase	2314
comp12936_c0	0.46	0.08	myosin heavy chain	2519
comp13080_c0	0.07	0.06	heparan sulfate proteoglycan 2	5821
comp13146_c0	8.83	5.49	contactin 3	1723
comp18562_c1	0.22	0.34	STAT5B	4131
comp14563_c0	0.65	0.37	Janus kinase	2192
comp19883_c1	0.16	0.33	early endosome antigen 1	4205
comp17906_c0	0.19	0.17	arginine-tRNA-protein transferase	2379
comp12697_c0	13.79	122.38	celD, endoglucanase	2314
comp16289_c0	1.55	10.10	Hsc70-3, heat shock 70 kDa protein	2283
comp18060_c1	2.5	45.63	matrix metalloproteinase	1102
comp18235_c0	0.64	5.25	glutathione S-transferase	1666
comp19645_c0	0.55	22.34	Glt1, glutamate synthase	7980
comp16672_c0	0.068	0.012	dual specificity phosphatase	1550
comp17631_c0	0.25	0.07	Dll4, Notch	3972
comp19506_c0	0.31	0.36	caspase, apoptosis-related cysteine protease	2366
comp3834_c0	1152.92	0.54	TAR1-A	1024
comp20427_c0	333.24	894.08	glutaminyl-peptide cyclotransferase	2430
comp20571_c0	94.21	93.93	carboxypeptidase A	3399
comp18765_c1	232	305	secreted frizzled-related protein 5	3110
comp20525_c0	97.42	3.19	lysosomal alpha-mannosidase	451
comp16349_c0	0.50	1.35	relish	2529

FC indicates the fold change; VA indicates *V. alginolyticus*.

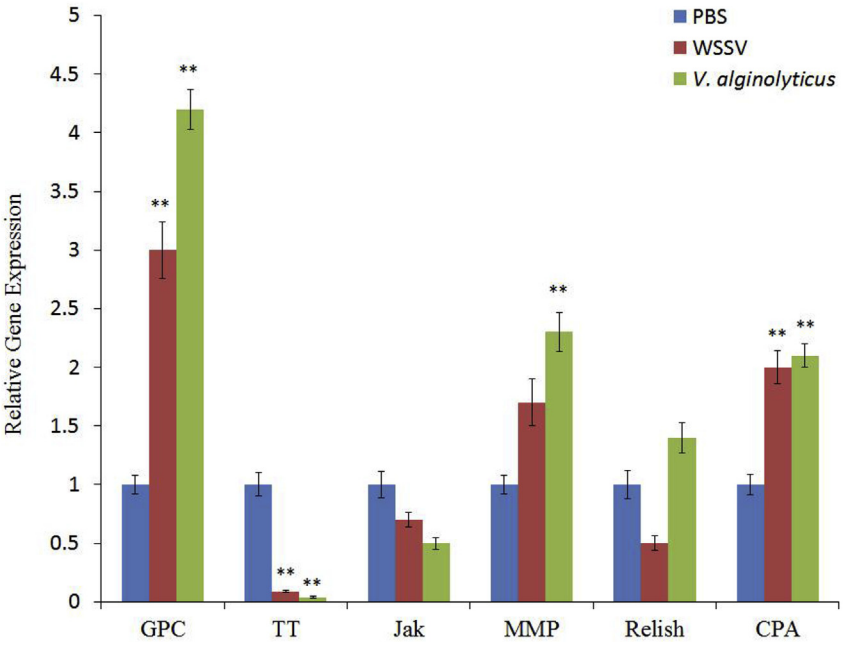


Fig. 9. The expression of the selected genes in *S. paramamosain* hemocytes in response to WSSV or *V. alginolyticus*. Data are shown as means ± SD (standard deviation) of three separate individuals in the tissues. Double asterisks indicate a significant difference ($P < 0.01$) between two samples. GPC indicates Glutaminyl-peptide cyclotransferase; TT indicates Troponin T; Jak indicates Janus kinase; MMP indicates Matrix metalloproteinase; CPA indicates Carboxypeptidase A.

Ethics statement

The animal subjects used in the present study are crab, which are invertebrates and are exempt from this requirement.

Conflicts of interest

There are no patents, products in development or marketed products to declare. This does not alter our adherence to all the gene policies on sharing data and materials.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.fsi.2018.06.003.

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