Aquaculture 560 (2022) 738563

Contents lists available at ScienceDirect

Aquaculture

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

Short communication

Transcriptome profiling of the Pacific oyster (*Crassostrea gigas*) suggests distinct host immune strategy in response to *Vibrio alginolyticus* infection

Yameng He [a], Xin Li [a], Chenyu Shi [a], Yin Li [a], Qi Li [a,b], Shikai Liu [a,b,*]

[a] Key Laboratory of Mariculture, Ministry of Education, Ocean University of China, Qingdao 266003, China
[b] Laboratory for Marine Fisheries Science and Food Production Processes, Qingdao National Laboratory for Marine Science and Technology, Qingdao 266237, China

ARTICLE INFO

Keywords:
Crassostrea gigas
Vibrio alginolyticus
Gill
RNA sequencing
Immune strategy

ABSTRACT

Bacterial infection is a serious challenge for oysters, which brings various disease problems to oyster culture. To better understand the immune response to bacterial infection, we performed RNA-seq to profile the transcriptome of gill tissue in the Pacific oyster (*Crassostrea gigas*) at short-term (6 h) and long-term (48 h) stages after *Vibrio alginolyticus* infection. A total of 1534 and 2483 differentially expressed genes (DEGs) were identified at short- and long-term stages after infection, respectively. Gene ontology (GO) analysis showed that the identified DEGs were significantly enriched in GO terms of stress response, ion transport, signal transduction and other biological processes. Notably, we observed that the immune strategy was altered in the *C. gigas* in response to *Vibrio* infection. In the short-term stage, most of the immune-related genes in *C. gigas* were down-regulated, and the immune response was low and may be suppressed. While in the long-term stage, large-scale immune-related genes associated with ion channels were activated and immune system was boosted. Distinct sets of immune-related genes with different functions were identified at short-term and long-term stages. Differences in these genes suggested the alteration of immune strategy in the *C. gigas* in response to *Vibrio* infection. This work provides valuable resources for better understanding of immune response to bacterial infection and will be important for disease monitoring and disease resistance breeding in the oysters.

1. Introduction

The Pacific oyster (*Crassostrea gigas*), naturally distributed in the northwest Pacific oceans, is an important aquaculture species around the world (Gagnaire et al., 2006; Peng et al., 2021; Troost, 2010; Zhang et al., 2019a). As an intertidal sessile organism, the oysters are subject to various biological challenges and environmental stresses (Forrest et al., 2009; Wang et al., 2019). In recent years, the oyster industry has frequently encountered mass mortality during summer season, which caused serious economic loss worldwide (Soletchnik et al., 2005). Numerous investigations on potential stressors and pathogens for mass summer mortality suggests a complicated process involved in both environmental stressors and pathogens, such as virus (*Ostreid herpesvirus 1*, OsHV-1) and bacteria (*Vibrio* spp.) (King et al., 2019; Segarra et al., 2010). *Vibrio* is a common class of bacteria in the marine environment (Drake et al., 2007). Among which, *V. alginolyticus* has been associated with mortality events in many marine organisms (Rameshkumar et al., 2017; Xie et al., 2020). Recently, *V. alginolyticus* was identified as an infectious pathogen causing mass mortality of *C. gigas* cultured in northern China during summer (Wang et al., 2021; Yang et al., 2021).

Oysters defend against pathogen infection with innate immune system same as other invertebrates (Song et al., 2010). Through innate immune response, immune cells recognize invading pathogens through pattern recognition receptors (PRRs), and then trigger a variety of downstream effector cells to mediate immune responses (Iwasaki and Medzhitov, 2015). Ion channels are pore-forming membrane proteins that play a critical role in the immune system (Feske et al., 2015; Stokes et al., 2016). They mediate the movement of various ions into and out of the plasma membrane and participate in basic physiological processes such as action potential conduction (Kondratskyi et al., 2018). For example, the effect of voltage-gated sodium channels (Nav) and their potential sub-types on the variability of paralytic shellfish toxins (PST) accumulation in the oysters was investigated to find that blocking of Nav by PST could trigger the activation of regulatory pathways to regulate the expression of Nav channels in *C. gigas* (Boullot et al., 2017). In addition, ion channels can also regulate various physiological functions of immune cells such as gene expression and apoptosis (Bose et al., 2015).

* Corresponding author at: Key Laboratory of Mariculture, Ministry of Education, Ocean University of China, Qingdao 266003, China.
E-mail address: liushk@ouc.edu.cn (S. Liu).

https://doi.org/10.1016/j.aquaculture.2022.738563
Received 20 April 2022; Received in revised form 27 May 2022; Accepted 27 June 2022
Available online 30 June 2022

Complex immune defense during bacterial challenge might disrupt metabolic homeostasis to fuel host defense (Erk et al., 2011; Rőszer, 2014). Transcriptome sequencing has been widely used to understand immune response at transcriptome level in bivalves (Metzker, 2010; Wang et al., 2009). For example, RNA sequencing (RNA-seq) was used to identify immune-related genes and pathways in *Mytilus* (Dong et al., 2017). In the oysters, many RNA-seq studies have been carried out to explore immune regulation, including virus infection (He et al., 2015), bacterial stimulation (Zhang et al., 2019b), heat shock stress (Yang et al., 2017), and heavy metal stress (Qiu et al., 2020). As the filter-feeding organ of mollusks, the immune effectors produced by the epithelial cells of gills can defend against pathogen invasion and participate in the immune response (Bachère et al., 2015). In addition, the gills of oysters have been identified as potential hematopoietic sites (Jemaa et al., 2014). Numerous studies with gill tissue in the oyster after exposure to pathogen have been conducted using molecular biology and omics approaches (Zhang et al., 2012; Kim et al., 2017).

In this study, RNA-Seq was conducted to analyze the transcriptome profiles of *C. gigas* at 6 h and 48 h after infection with *V. alginolyticus*. The objectives are to (1) identify DEGs and pathways involved in host immune response, and (2) explore immune strategies against bacterial infection between short-term and long-term stages. This work would provide valuable resources for further understanding the innate immune defense system and will be important for disease monitoring and disease resistance breeding in the oysters.

2. Materials and methods

2.1. Experiment animals and bacterial infection

Animals used for experiment in this study were two-year old Pacific oysters which were healthy without infection after diagnosis (Table S1). Before the experiment, the oysters were acclimatized in a 50 L glass tank with filtered seawater (23 °C, salinity 30‰) for a week. During the period of acclimation, the seawater was consecutively aerated and changed daily, and the oysters were fed with Chlorella ad libitum. The bacterial pathogen used was *V. alginolyticus* Cg5 strain that was previously isolated by our group (Yang et al., 2021). A total of 50*C. gigas* were used for the bacterial infection experiment. After the oysters were anesthetized in magnesium chloride ($MgCl_2$, 50 g/L) solution, 100 μL of *V. alginolyticus* suspension (5×10^7 CFU/mL) was injected into the adductor muscle of each oyster with a microinjector (100 ± 0.5 μL). During injection, the centrifuge tubes were slowly turned over at regular intervals so that the bacterial suspensions were well-mixed during the bacterial challenge. After injection, the oysters were randomly divided into three groups as replicates and cultured in the filtered seawater (23 °C, salinity 30‰) with same practice as mentioned above. Gill tissues were collected from six oysters (two individuals per group) at 0 h, 6 h and 48 h post infection, respectively. Equal amount of the tissues from the two individuals were pooled together as one sample to generate three biological replicates at each time point. All samples were stored in −80 °C freezer until use.

2.2. RNA extraction, library construction and sequencing

The gill tissue from each sample was ground into powder in liquid nitrogen followed by extraction of total RNA using Trizol reagent (Invitrogen) according to the manufacturer's instructions. The RNA quality and concentration were measured by running 1% agarose gel electrophoresis and Nanodrop (Thermo Fisher Scientific). Nine sequencing libraries were constructed using the NebNext®Ultra™ RNA Library Prep Kit for Illumina® (NEB, USA) for samples collected at three time-points, including 0 h, 6 h and 48 h. The sample taken at 0 h were used as the control group. After evaluation of the library quality, sequencing was performed using Illumina NovaSeq 6000 system for 150 bp paired-end reads.

2.3. Bioinformatics analysis

Quality control of raw reads obtained from high-throughput sequencer was performed using FastQC (Thrash et al., 2018). The raw reads were trimmed to obtain high-quality clean reads. The paired-end clean reads were then aligned to the indexed reference genome (GCA_902806645.1) using Hisat2 (v2.0.5) (Peñaloza et al., 2021; Kim et al., 2015). Multiple testing correction was carried out using Benjamini-Hochberg method. Differentially expressed genes (DEGs) were defined as the adjusted *P*-value <0.05 and $|\log_2(\text{FoldChange})| > 1$. The DESeq2 was performed to identify DEGs among samples at different time-points compared with the control group (Love et al., 2014). The ClusterProfiler was used for Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analysis of DEGs.

2.4. Validation using quantitative real-time PCR

To verify the reliability of RNA-seq data, we selected 10 representative immune-related genes for quantitative real-time PCR (qRT-PCR) analysis. The *EF-1α* (elongation factor 1α) was used as the reference gene for internal standardization. The primers were designed by Primer Premier 5 (Table S2). RNA samples used for qRT-PCR were same as those used for RNA-Seq analysis. In brief, RNA samples were reversed transcribed using a cDNA synthesis kit (TAKARA). SYBR Green qRT-PCR was performed as previously described (Yang et al., 2012). Finally, the relative gene expression levels were calculated using $2^{-\Delta\Delta CT}$ (Livak and Schmittgen, 2001).

3. Results

3.1. RNA-seq of C. gigas infected with Vibrio bacteria

A total of 64.52 Gb raw reads were obtained by sequencing nine libraries constructed from the gill of *C. gigas* after *Vibrio* infection, including 19.00 Gb at 0 h, 23.49 Gb at 6 h and 22.03 Gb at 48 h. Over 94% of the raw sequencing bases had a Q-score ≥ 30. After quality control and trimming of low-quality reads, the clean reads of each library were mapped to the oyster genome with mapping rate ranging from 76% to 83% (Table S3).

3.2. Identification of differentially expressed genes (DEGs)

In order to determine the transcriptome profile altered by the *V. alginolyticus* infection, we detected DEGs at 6 h and 48 h compared with 0 h. A total of 1534 (6 h) and 2483 (48 h) DEGs were identified, respectively. Ten immune-related genes with high relative expression levels were selected for qRT-PCR analysis to confirm the reliability of RNA-seq analysis (Fig. 1). Among the DEGs identified at short-term stage (6 h), 509 genes were up-regulated and 1025 genes were down-regulated. While at long-term stage (48 h), 937 genes were up-regulated and 1546 genes were down-regulated (Fig. 2). A great difference in the number of DEGs was observed between the two stages, indicating that significant transcriptome changes may have occurred along the process of infection with *V. alginolyticus*.

3.3. Functional analysis based on GO and KEGG annotations

Gene Ontology (GO) annotation and enrichment analysis of DEGs allowed identification of enriched GO terms (Table S4). The short-term (6 h) DEGs were significantly enriched in biological processes including substance metabolism, stress response, ion transport, apoptosis and physiological response. In the long-term (48 h), cellular processes related to translation activity, oxidative stress, ion homeostasis and ion channels were significantly enriched. Moreover, the processes related to transcription and signal transduction were also highly represented.

To further determine gene pathways that were involved in the

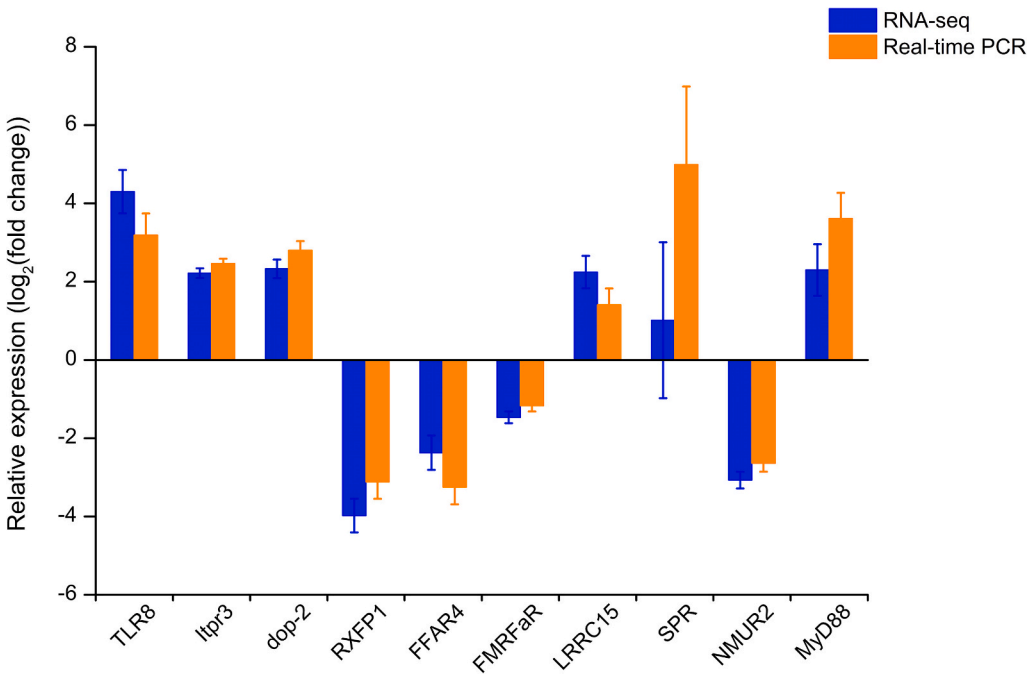

Fig. 1. Comparison of relative expression levels determined by RNA-seq and qRT-PCR. *EF-1α* gene was used as internal control. Orange bars denote Real time-PCR data, blue bars indicate RNA-seq data. Vertical bars represent the mean ± S.D. ($n = 3$). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

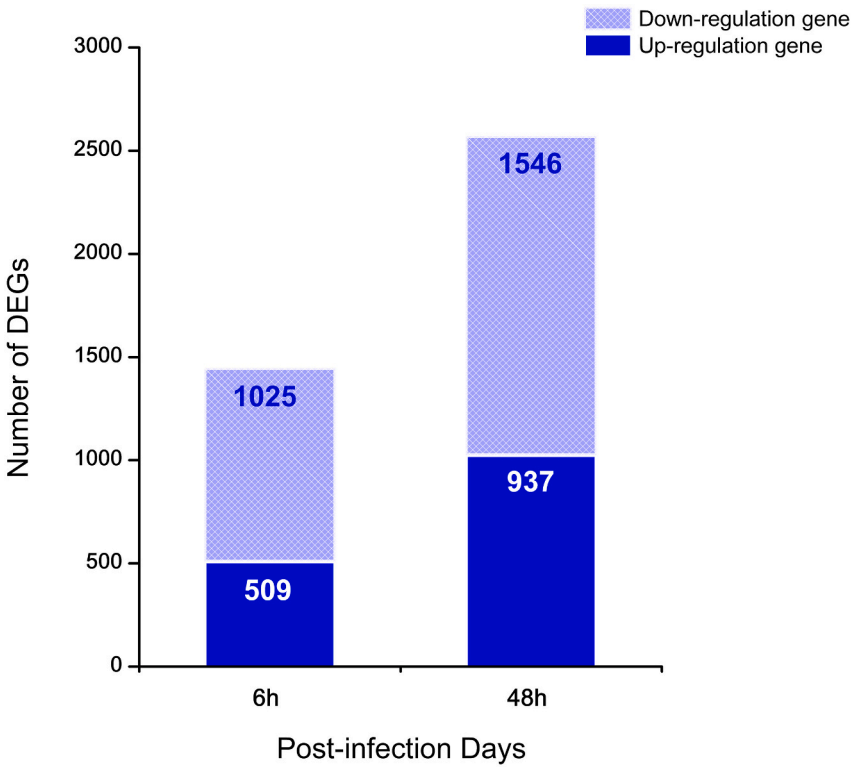

Fig. 2. DEGs between the treatment and control Pacific oysters ($p < 0.05$). The horizontal and vertical axis represents post-infection days and number of DEGs, respectively.

response to bacterial infection, we performed KEGG enrichment analysis of up-regulated and down-regulated genes in the short-term (6 h) and long-term (48 h) stage, respectively (Fig. 3, Table S5). The up-regulated genes in both stages were significantly enriched in "Ion channels", "Inflammatory mediator regulation of TRP channels" and "Nod-like receptor signaling pathway". In contrast, "G protein-coupled receptors" was the most significant KEGG pathway of short-term (6 h) down-regulated genes, while for the long-term (48 h) down-regulated genes, the "Translation factor" and "MAPK signaling pathway" are the most significantly enriched pathways, respectively. Genes in these pathways

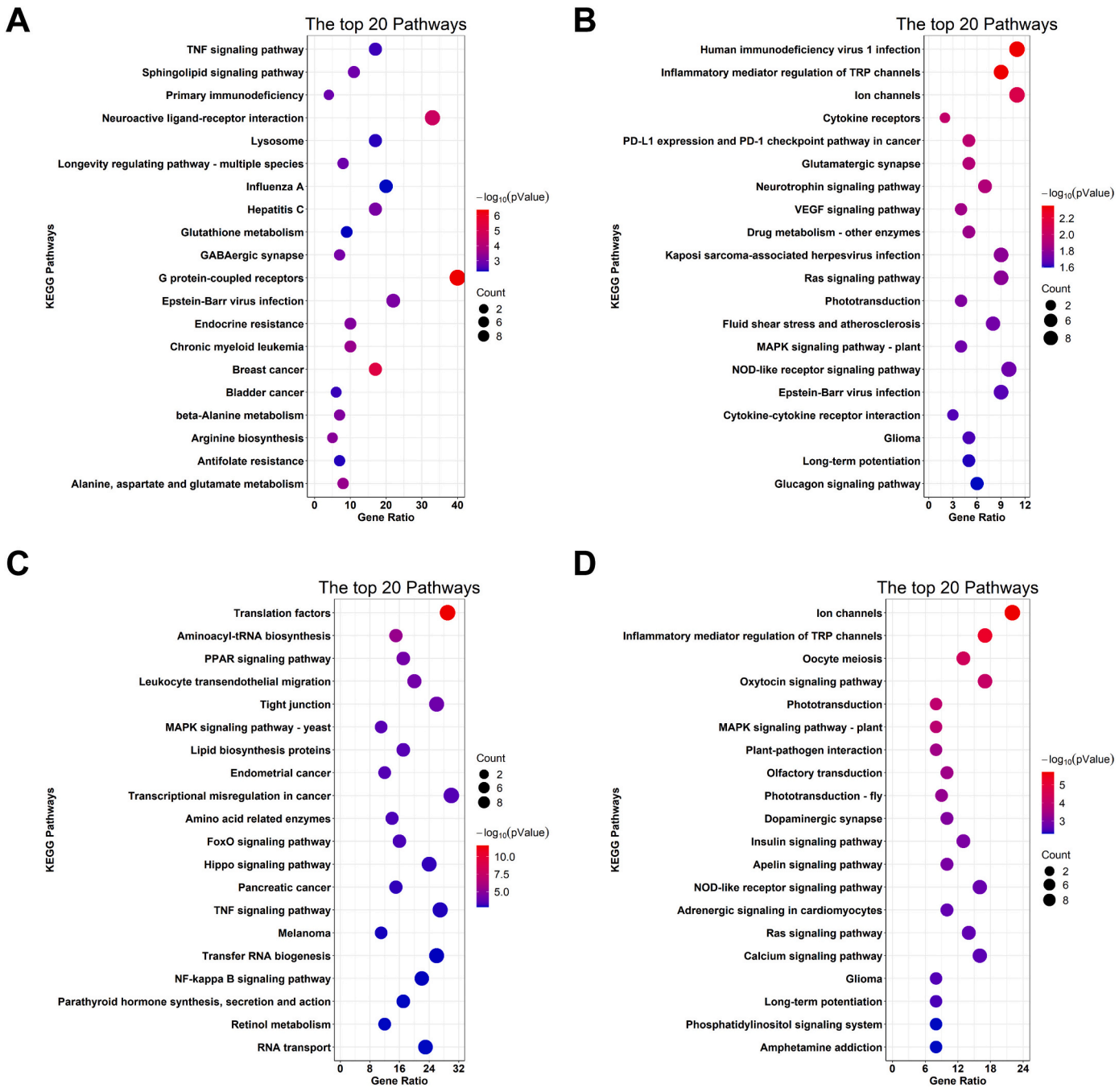

Fig. 3. Summary of Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analysis of DEGs in the short-term (6 h) and long-term (48 h) stage after infection. (A) KEGG enrichment of short-term (6 h) up-regulated genes; (B) KEGG enrichment of short-term (6 h) down-regulated genes; (C) KEGG enrichment of long-term (48 h) up-regulated genes; (D) KEGG enrichment of long-term (48 h) down-regulated genes.

were annotated, which were mostly TRP family genes and calmodulin, and others related to innate immune response, such as TNF receptor-associated factor and Baculoviral IAP repeat-containing protein.

3.4. Distinct immune response between short- and long-term stage in *C. gigas* upon *Vibrio* infection

In order to explore the immune changes of *C. gigas* upon infection with *V. alginolyticus*, we conducted further analysis on the annotated GO pathway. It was found that a large number of genes showed a downward trend in the short-term (6 h) (Fig. 4A, Table S4), which were involved in various biological processes including "drug metabolic process", "positive regulation of inflammatory response" and "leukocyte apoptotic process". This indicates that the immunity of *C. gigas* may be inhibited in the short term under *V. alginolyticus* infection. Notably, pathways with significantly high expression of DEGs accounted for about half of all GO entries in the long-term (48 h) (Fig. 4B, Table S4). Some of which include "transition metal ion transmembrane transporter activity", and "calcium-release channel activity". Interestingly, the significantly up-regulated genes were mainly related to ion channels in the long-term stage (48 h), while these genes were very few in the short-term stage (6 h). These results suggest that during different stages of infection by *V. alginolyticus*, the immune response of *C. gigas* can be enhanced or suppressed for regulation.

In order to determine the immune strategy of *C. gigas* against *Vibrio*, we selected a set of critical genes with distinct known functions for

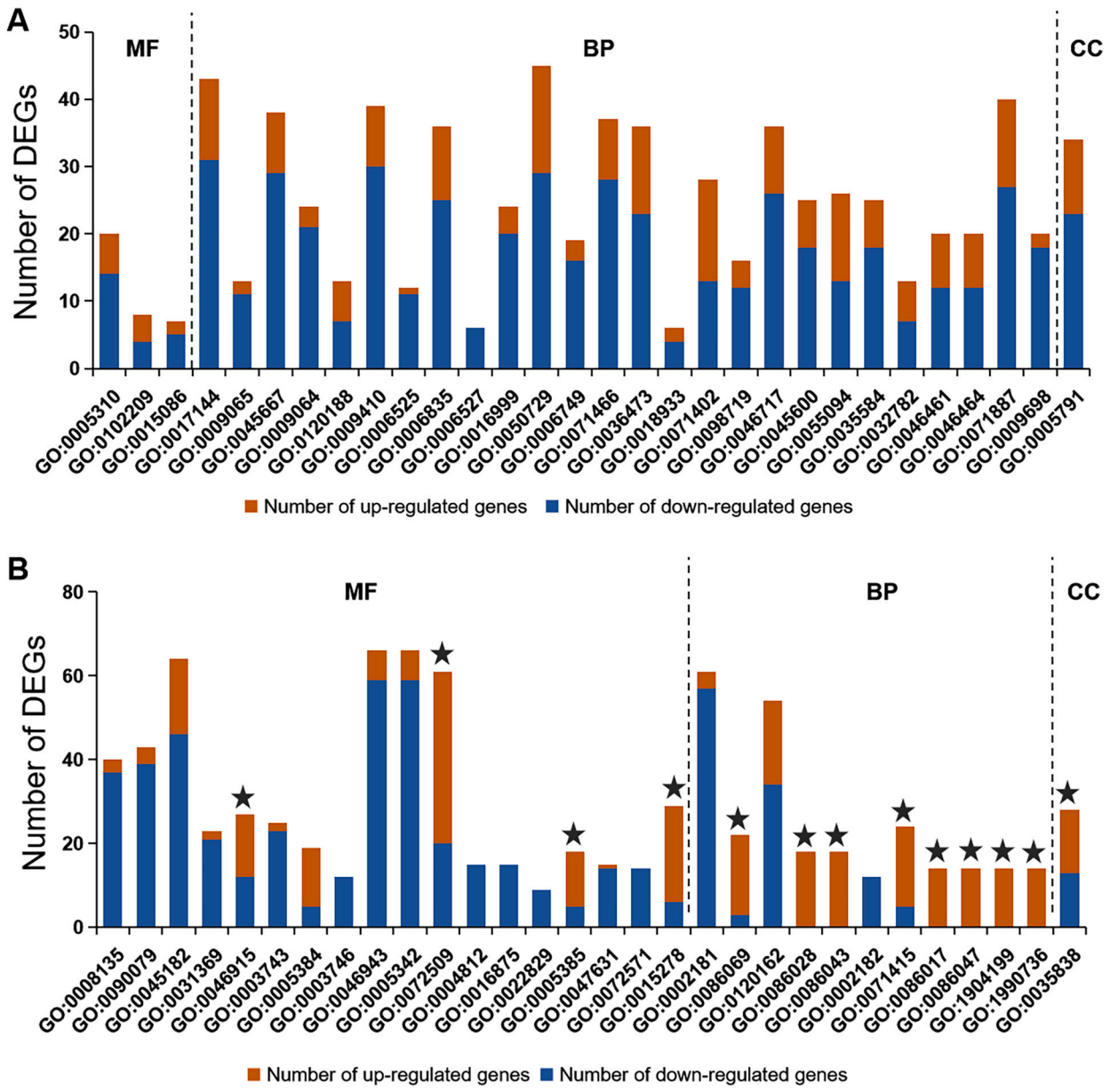

Fig. 4. GO enrichment analysis of DEGs in the short-term (6 h) and long-term (48 h) stage after infection. (A) the short-time (6 h) stage and (B) the long-term (48 h) stage. The horizontal axis represents the GO pathway, the vertical axis represents the number of DEGs, the bar of orange represents the up-regulated genes, and the bar of blue represents the down-regulated genes. Pathways with more up-regulated genes than down-regulated genes are marked with asterisks. Only the top 30 enriched pathway terms are shown. The detailed GO entries were provided in the supplementary table.

verification. The results show that most of the tested genes associated with apoptotic process and inflammatory response were expressed at low levels in the short-term stage, while most of the tested genes involved in innate immune response and ion transport were highly expressed in the long-term stage (Fig. 5, Table S6). The down-regulation of the tested genes indicates that the immune response of oysters is suppressed, while up-regulation of genes suggests the stimulation of immune defense system after the infection of *V. alginolyticus* in the *C. gigas*. These were consistent with our speculation that *C. gigas* could respond to *V. alginolyticus* infection with different immune strategy in an acute or chronic manner.

4. Discussion

Vibrios are reported as opportunistic pathogens that cause high mortality of oysters (Paillard et al., 2004), which pose a great threat to oyster aquaculture industry. In the past, there have been many studies on *Vibrio* infection in oysters to assess the immune response (Chen et al., 2021; Labreuche et al., 2006; Zhang et al., 2014). These studies have promoted the understanding of bacterial stress, but the regulation mechanism of immune response in oyster remains to be further explored. In this study, the pathogenic *V. alginolyticus* was used to infect the *C. gigas*, and RNA-seq was performed to analyze the transcriptome response to pathogen infection. A total of 1534 and 2483 DEGs were identified at the short-term (6 h) and the long-term (48 h) after bacterial infection, respectively. Compared with the short-term stage, the number of DEGs increased significantly in the long-term stage. The increased number of DEGs should be attributable to immune response to the bacterial infection. The transcriptome profile reflects the complex interaction between the host immune system and pathogens.

Functional analysis of the DEGs suggested their involvement in a variety of biological processes. Specifically, genes associated with the

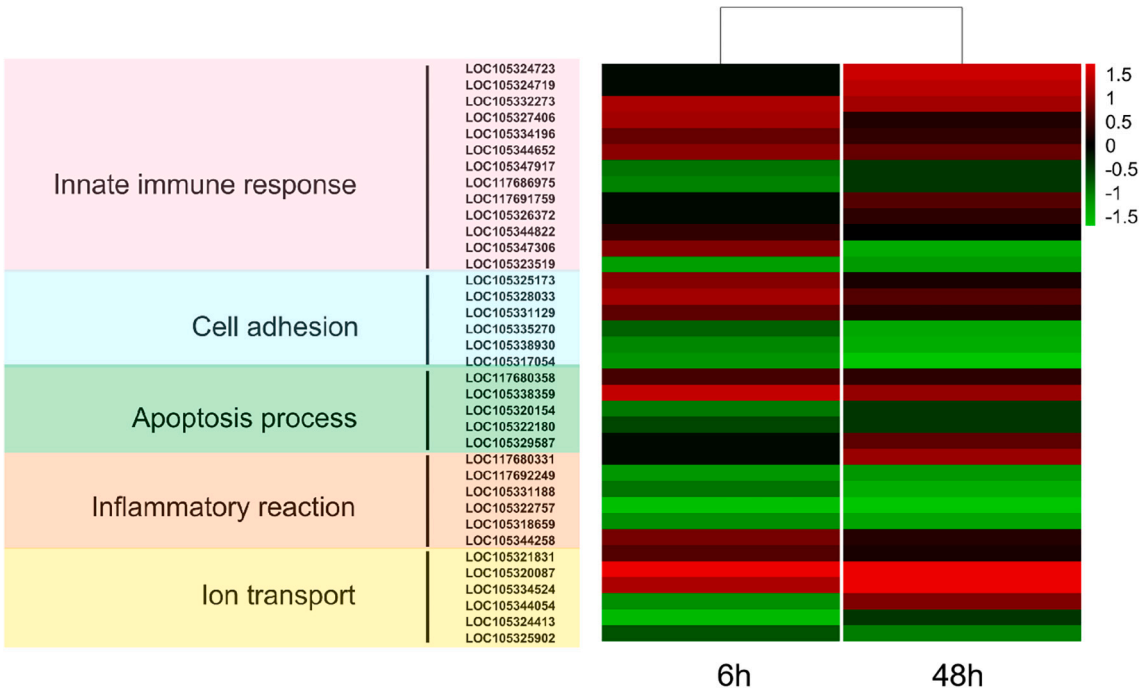

Fig. 5. Heat map of selected genes with different known functions identified at short- and long-term stages. The intensity of the colour from green to red indicates the magnitude of differential expression. Red and green indicate up- and down-regulation, respectively. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

immune system and substance metabolism were significantly expressed in the short-term stage, such as "drug metabolic process" and "positive regulation of inflammatory response" (Table S4). In the long-term stage, DEGs were significantly enriched in biological processes related to translation activity, ion transport and signal transduction (Table S4), including "translation factor activity, RNA binding", "cytoplasmic translation" and "transition metal ion transmembrane transporter activity". The transcription and translation regulation of gene expression plays critical roles in organisms (Thach, 1992). Pathogens have been reported to suppress host defense and stress responses by disrupting translation initiation in host cells (Batool et al., 2021). The enrichment of GO terms revealed these molecular events that were involved in the immune response of oysters after *Vibrio* infection.

Upon bacterial infection, many genes show down-regulated expression patterns in a certain period in response to immune challenges (Zhang et al., 2019a). Some immune genes have been reported to be significantly down-regulated during brief periods of stress in oysters in response to pesticide and bacterial infections (Gagnaire et al., 2007). This indicated that the infection of *V. alginolyticus* had a serious impact on the normal immune response of oysters, and might lead to the decreased immunity under short-term infection stage (Zhang et al., 2016). In this study, the distinct immune strategy of oyster was found to defend against bacterial infection through further analysis of GO terms and KEGG pathways. In the short-term stage (6 h), most of the DEGs showed suppressed expression pattern, which was similar to the previous observations (Zhang et al., 2019b; Gagnaire et al., 2007). This may result from the suppression of the immune response of *C. gigas* in the early stage, which was more conducive to the proliferation of bacteria. In the long-term stage (48 h), most DEGs were up-regulated with GO entries mostly associated with ion transport and ion channels. This indicates that during long-term stage, the *C. gigas* has adopted possible cell reprogramming to promote immune adaptation and suppress bacterial infections. Ion channels and transporters mediate the movement of charged ions into and out of the plasma membrane (Stokes et al., 2016). In immune cells, calcium (Ca^{2+}), magnesium (Mg^{2+}), and zinc (Zn^{2+}) play important roles in regulating intracellular signaling pathways (Feske et al., 2012; Feske et al., 2015). Wang et al. revealed the response of calcium signaling pathways in oysters under environmental stress, indicating that calcium ion homeostasis was extremely important in the oyster immunity (Wang et al., 2020). Furthermore, it is reported that the higher level of Cu/Zn the oyster accumulated, the stronger antibacterial ability was shown (Shi et al., 2019). These results indicate that with the increase of stress time, organisms regulate gene expression of immune cells by effectively adjusting the intracellular concentration of various ions in vivo in order to improve the defense ability against *Vibrio*.

Ion channels are a class of membrane proteins that control various molecular signaling events in many different cell types (Stokes et al., 2016). Transient receptor potential (TRP) channels are cation channels that facilitate Ca^{2+} permeability (Nilius and Voets, 2005). By maintaining intracellular calcium homeostasis, TRPs regulate immune cell functions including the production of inflammatory mediators (Khalil et al., 2018). In this study, both short-term and long-term up-regulated genes were significantly enriched in two KEGG enrichment pathways, including inflammatory mediator regulation of TRP channels and Ion channels. The expression levels of transient receptor potential cation channel subfamily M member-like 2 and calmodulin were significantly up-regulated (Fig. 6, Table S7). Transient receptor potential melastatin 2 (TRPM2) is a Ca^{2+} permeable cation channel required for immune cell activation (Mortadza et al., 2015). Gated by binding to ADP-ribose, a metabolite formed when cells are exposed to Reactive oxygen species (ROS). ROS can promote bacterial killing and play a crucial role in host immune defense against pathogens (Knowles et al., 2011). It has been reported that TRPM2 may contribute to maintaining ROS response and apoptosis homeostasis in *C. gigas* (Fu et al., 2021). This suggests that ion channels play a crucial role in the immune response of *C. gigas*. Verification of different functional immunity genes revealed differences in the basal expression levels of these genes, indicating different immune status between short-term and long-term stages after infection. These differences may suggest the distinct immune strategy of *C. gigas* in response to *V. alginolyticus* infection.

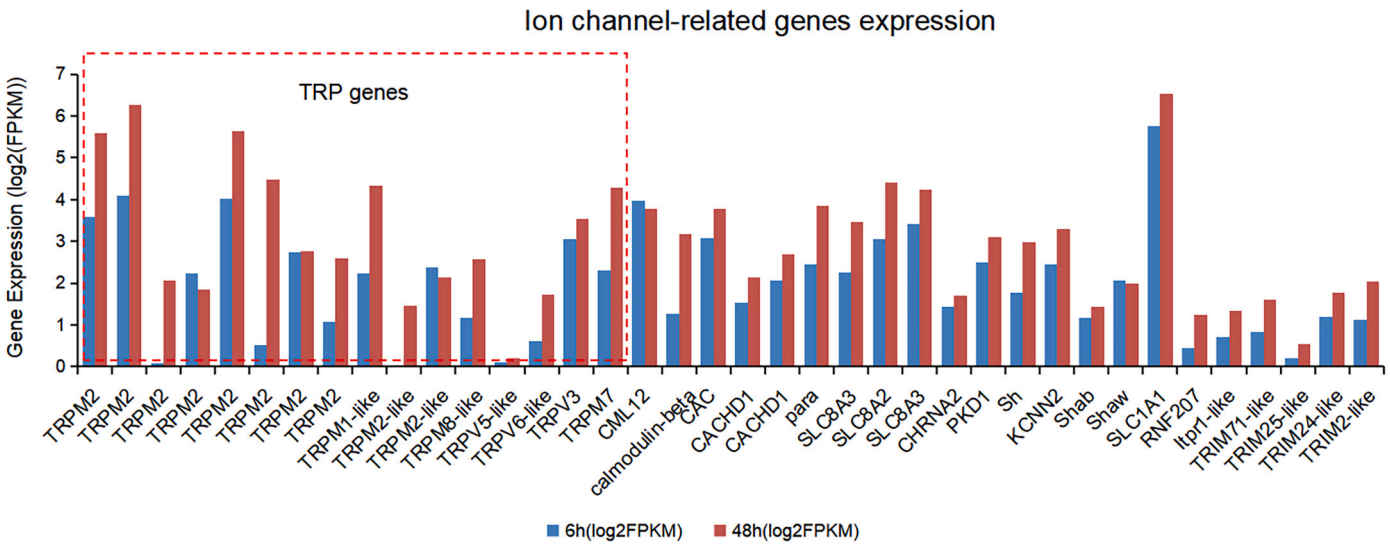

Fig. 6. Expression of ion channel-related genes at the short-term (6 h) and long-term (48 h) stage after infection. Detailed information of the genes was provided in the supplementary table.

5. Conclusion

To explore the molecular basis of oyster response to pathogen infection, we performed transcriptome profiling of *C. gigas* infected by *V. alginolyticus*. Functional enrichment analysis of the DEGs revealed enriched biological processes including stress response, ion transport, and signal transduction. KEGG pathway analysis also suggested that ion channels, inflammatory response and apoptotic system played critical roles in the *C. gigas* immune response. This study revealed the distinct immune strategy of *C. gigas* against *V. alginolyticus* infection at different stages. The immune-related genes with different functions were induced or suppressed dynamically during the infection, showing distinct expression pattern between short-term and long-term stages. This study provides valuable resource for understanding of immune response against *Vibrio* infection in the oysters, which should be of great significance for disease surveillance, and disease resistance breeding in the oysters.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.aquaculture.2022.738563.

Credit author statement

Yameng He: Investigation, Methodology, Data curation, Formal analysis, Writing-original draft.

Xin Li: Investigation, Data curation and Validation.

Chenyu Shi & Yin Li: Formal analysis, Validation and Visualization.

Qi Li: Resources.

Shikai Liu: Conceptualization, Supervision, Data curation, Funding acquisition, Writing- review editing.

Declaration of Competing Interest

We declare that there are no conflicts of interest.

Acknowledgments

This work was funded by the Young Talent Program of Ocean University of China (No. 201812013), and the Fundamental Research Funds for the Central Universities (No. 20204201).

References

Bachère, E., Rosa, R.D., Schmitt, P., Poirier, A.C., Merou, N., Charrière, G.M., Destoumieux-Garzón, D., 2015. The new insights into the oyster antimicrobial defense: cellular, molecular and genetic view. Fish Shellfish Immunol. 46, 50–64.

Batool, W., Shabbir, A., Lin, L.L., Chen, X.M., An, Q.L., He, X.J., Norvienyeku, J., 2021. Translation initiation factor eIF4E positively modulates conidiogenesis, appressorium formation, host invasion and stress homeostasis in the filamentous fungi *Magnaporthe oryzae*. Front. Plant Sci. 12, 1170.

Bose, T., Cieślar-Pobuda, A., Wiechec, E., 2015. Role of ion channels in regulating Ca^{2+} homeostasis during the interplay between immune and cancer cells. Cell Death Dis. 6, e1648.

Boullot, F., Castrec, J., Bidault, A., Dantas, N., Payton, L., Perrigault, M., Soudant, P., 2017. Molecular characterization of voltage-gated sodium channels and their relations with paralytic shellfish toxin bioaccumulation in the Pacific oyster *Crassostrea gigas*. Mar. Drugs. 15, 21.

Chen, H.L., Cai, X.H., Qiu, H.N., Fang, J., Wu, X.Z., 2021. A novel C-type lectin from *Crassostrea gigas* involved in the innate defense against *Vibrio alginolyticus*. Biochem. Biophys. Res. Commun. 566, 155–163.

Dong, W.Q., Chen, Y.X., Lu, W.X., Wu, B., Qi, P.Z., 2017. Transcriptome analysis of *Mytilus coruscus* hemocytes in response to *Vibrio alginnolyficus* infection. Fish Shellfish Immunol. 70, 560–567.

Drake, S.L., DePaola, A., Jaykus, L.A., 2007. An overview of *Vibrio vulnificus* and *Vibrio parahaemolyticus*. Compr. Rev. Food Sci. F. 6, 120–144.

Erk, M., Ivanković, D., Strižak, Ž., 2011. Cellular energy allocation in mussels (*Mytilus galloprovincialis*) from the stratified estuary as a physiological biomarker. Mar. Pollut. Bull. 62, 1124–1129.

Feske, S., Skolnik, E.Y., Prakriya, M., 2012. Ion channels and transporters in lymphocyte function and immunity. Nat. Rev. Immunol. 12, 532–547.

Feske, S., Wulff, H., Skolnik, E.Y., 2015. Ion channels in innate and adaptive immunity. Rev. Immunol. 33, 291–353.

Forrest, B.M., Keeley, N.B., Hopkins, G.A., Webb, S.C., Clement, D.M., 2009. Bivalve aquaculture in estuaries: review and synthesis of oyster cultivation effects. Aquaculture. 298, 1–15.

Fu, H.R., Jiao, Z.X., Li, Y.J., Tian, J., Ren, L.T., Zhang, F.Q., Liu, S.K., 2021. Transient receptor potential (TRP) channels in the Pacific oyster (*Crassostrea gigas*): genome-wide identification and expression profiling after heat stress between *C. gigas* and *C. angulata*. Int. J. Mol. Sci. 22, 3222.

Gagnaire, B., Frouin, H., Moreau, K., Thomas-Guyon, H., Renault, T., 2006. Effects of temperature and salinity on haemocyte activities of the Pacific oyster, *Crassostrea gigas* (Thunberg). Fish Shellfish Immunol. 20, 536–547.

Gagnaire, B., Gay, M., Huvet, A., Daniel, J.Y., Saulnier, D., Renault, T., 2007. Combination of a pesticide exposure and a bacterial challenge: in vivo effects on immune response of Pacific oyster, *Crassostrea gigas* (Thunberg). Aquat. Toxicol. 84, 92–102.

He, Y., Jouaux, A., Ford, S.E., Lelong, C., Sourdaine, P., Mathieu, M., Guo, X., 2015. Transcriptome analysis reveals strong and complex antiviral response in a mollusc. Fish Shellfish Immunol. 46, 131–144.

Iwasaki, A., Medzhitov, R., 2015. Control of adaptive immunity by the innate immune system. Nat. Immunol. 16, 343–353.

Jemaa, M., Morin, N., Cavelier, P., Cau, J., Strub, J.M., Delsert, C., 2014. Adult somatic progenitor cells and hematopoiesis in oysters. J. Exp. Biol. 217, 3067–3077.

Khalil, M., Alliger, K., Weidinger, C., Yerinde, C., Wirtz, S., Becker, C., Engel, M.A., 2018. Functional role of transient receptor potential channels in immune cells and epithelia. Front. Immunol. 9, 174.

Kim, D., Langmead, B., Salzberg, S.L., 2015. HISAT: a fast spliced aligner with low memory requirements. Nat. Methods 12, 357–360.
Kim, B.M., Kim, K., Choi, I.Y., Rhee, J.S., 2017. Transcriptome response of the Pacific oyster, *Crassostrea gigas* susceptible to thermal stress: a comparison with the response of tolerant oyster. Mol. Cell. Toxicol. 13, 105–113.
King, W.L., Jenkins, C., Go, J., Siboni, N., Seymour, J.R., Labbate, M., 2019. Characterisation of the Pacific oyster microbiome during a summer mortality event. Microb. Ecol. 77, 502–512.
Knowles, H., Heizer, J.W., Li, Y., Chapman, K., Ogden, C.A., Andreasen, K., Humann, J., 2011. Transient receptor potential Melastatin 2 (TRPM2) ion channel is required for innate immunity against *Listeria monocytogenes*. PNAS. 108, 11578–11583.
Kondratskyi, A., Kondratska, K., Skryma, R., Klionsky, D.J., Prevarskaya, N., 2018. Ion channels in the regulation of autophagy. Autophagy. 14, 3–21.
Labreuche, Y., Lambert, C., Soudant, P., Boulo, V., Huvet, A., Nicolas, J.L., 2006. Cellular and molecular hemocyte responses of the Pacific oyster, *Crassostrea gigas*, following bacterial infection with *Vibrio aestuarianus* strain 01/32. Microbes Infect. 8, 2715–2724.
Livak, K.J., Schmittgen, T.D., 2001. Analysis of relative gene expression data using real-time quantitative PCR and the 2− ΔΔCT method. Methods. 25, 402–408.
Love, M., Anders, S., Huber, W., 2014. Differential analysis of count data–the DESeq2 package. Genome Biol. 15, 10–1186.
Metzker, M.L., 2010. Sequencing technologies—the next generation. Nat. Rev. Genet. 11, 31–46.
Mortadza, S.A.S., Wang, L., Li, D.L., Jiang, L., 2015. TRPM2 channel-mediated ROS-sensitive Ca^{2+} signaling mechanisms in immune cells. Front. Immunol. 6, 407.
Nilius, B., Voets, T., 2005. TRP channels: a TR (I) P through a world of multifunctional cation channels. Pflugers Arch. 451, 1–10.
Paillard, C., Roux, F.L., Borrego, J.J., 2004. Bacterial disease in marine bivalves, a review of recent studies: trends and evolution. Aquat. Living Resour. 17, 477–498.
Peñaloza, C., Gutierrez, A.P., Eöry, L., Wang, S., Guo, X.M., Archibald, A.L., Houston, R. D., 2021. A chromosome-level genome assembly for the Pacific oyster *Crassostrea gigas*. GigaScience. 10, 3.
Peng, D.M., Zhang, S.C., Zhang, H.Z., Pang, D.Z., Yang, Q., Jiang, R.H., Zhu, Y.G., 2021. The oyster fishery in China: trend, concerns and solutions. Mar. Policy 129, 104524.
Qiu, L.M., Chen, H., Zhou, Z., Zhang, H., Liu, R., Yi, Q.L., Wang, L.L., 2020. Transcriptomic profile of oyster *Crassostrea gigas* hemocyte after short-term cadmium exposure and bacteria stimulation. Fish Shellfish Immunol. 98, 138–146.
Rameshkumar, P., Nazar, A.K.A., Pradeep, M.A., Kalidas, C., Jayakumar, R., Tamilmani, G., Venkatesan, V., 2017. Isolation and characterization of pathogenic *Vibrio alginolyticus* from sea cage cultured cobia (*Rachycentron canadum* (Linnaeus 1766)) in India. Lett. Appl. Microbiol. 65, 423–430.
Rőszer, T., 2014. The invertebrate midintestinal gland ("hepatopancreas") is an evolutionary forerunner in the integration of immunity and metabolism. Cell Tissue Res. 358, 685–695.
Segarra, A., Pépin, J.F., Arzul, I., Morga, B., Faury, N., Renault, T., 2010. Detection and description of a particular Ostreid herpesvirus 1 genotype associated with massive mortality outbreaks of Pacific oysters, *Crassostrea gigas*, in France in 2008. Virus Res. 153, 92–99.
Shi, B., Wang, T., Zeng, Z., Zhou, L., You, W.W., Ke, C.H., 2019. The role of copper and zinc accumulation in defense against bacterial pathogen in the Fujian oyster (*Crassostrea angulata*). Fish Shellfish Immunol. 92, 72–82.
Soletchnik, P., Lambert, C., Costil, K., 2005. Summer mortality of *Crassostrea gigas* (Thunberg) in relation to environmental rearing conditions. J. Shellfish Res. 24, 197–207.
Song, L.S., Wang, L.L., Qiu, L.M., Zhang, H., 2010. Bivalve immunity. Invertebr. Immun. 708, 44–65.
Stokes, L., MacKenzie, A.B., Sluyter, R., 2016. Roles of ion channels in immune cells. Front. Immunol. 7, 48.
Thach, R.E., 1992. Cap recap: the involvement of eIF-4F in regulating gene expression. Cell. 68, 177–180.
Thrash, A., Arick, I.I.M., Peterson, D.G., 2018. Quack: a quality assurance tool for high throughput sequence data. Anal. Biochem. 548, 38–43.
Troost, K., 2010. Causes and effects of a highly successful marine invasion: case-study of the introduced Pacific oyster *Crassostrea gigas* in continental NW European estuaries. J. Sea Res. 64, 145–165.
Wang, Z., Gerstein, M., Snyder, M., 2009. RNA-Seq: a revolutionary tool for transcriptomics. Nat. Rev. Genet. 10, 57–63.
Wang, L.L., Zhang, H., Wang, M.Q., Zhou, Z., Wang, W.L., Liu, R., Song, L.S., 2019. The transcriptomic expression of pattern recognition receptors: insight into molecular recognition of various invading pathogens in oyster *Crassostrea gigas*. Dev. Comp. Immunol. 91, 1–7.
Wang, X.D., Wang, M.Q., Wang, W.L., Liu, Z.Q., Xu, J.C., Jia, Z.H., Song, L.S., 2020. Transcriptional changes of Pacific oyster *Crassostrea gigas* reveal essential role of calcium signal pathway in response to CO_2-driven acidification. Sci. Total Environ. 741, 140177.
Wang, H.B., Yang, B., Li, X., Li, Q., Liu, S.K., 2021. Screening of bacterial pathogens associated with mass summer mortality of the Pacific oyster, *Crassostrea gigas*, in China. Aquac. Rep. 20, 100672.
Xie, J.S., Bu, L.F., Jin, S., Wang, X.Y., Zhao, Q.S., Zhou, S.M., Xu, Y.J., 2020. Outbreak of vibriosis caused by *Vibrio harveyi* and *Vibrio alginolyticus* in farmed seahorse *Hippocampus kuda* in China. Aquaculture. 523, 735168.
Yang, C.Y., Wang, L.L., Siva, V.S., Shi, X.W., Jiang, Q.F., Wang, J.J., Song, L.S., 2012. A novel cold-regulated cold shock domain containing protein from scallop *Chlamys farreri* with nucleic acid-binding activity. PLoS One 7, e32012.
Yang, C.Y., Gao, Q., Liu, C., Wang, L.L., Zhou, Z., Gong, C.H., Song, L.S., 2017. The transcriptional response of the Pacific oyster *Crassostrea gigas* against acute heat stress. Fish Shellfish Immunol. 68, 132–143.
Yang, B., Zhai, S.Y., Li, X., Tian, J., Li, Q., Shan, H.W., Liu, S.K., 2021. Identification of *Vibrio alginolyticus* as a causative pathogen associated with mass summer mortality of the Pacific oyster (*Crassostrea gigas*) in China. Aquaculture. 535, 736363.
Zhang, L.L., Hou, R., Su, H.L., Hu, X.L., Wang, S., Bao, Z.M., 2012. Network analysis of oyster transcriptome revealed a cascade of cellular responses during recovery after heat shock. PLoS One 7, e35484.
Zhang, T., Qiu, L.M., Sun, Z.B., Wang, L.L., Zhou, Z., Liu, R., Song, L.S., 2014. The specifically enhanced cellular immune responses in Pacific oyster (*Crassostrea gigas*) against secondary challenge with *Vibrio splendidus*. Dev. Comp. Immunol. 45, 141–150.
Zhang, G.F., Li, L., Meng, J., Qi, H.G., Qu, T., Xu, F., Zhang, L.L., 2016. Molecular basis for adaptation of oysters to stressful marine intertidal environments. Ann. Rev. Anim. Biosci. 4, 357–381.
Zhang, F.Q., Hu, B.Y., Fu, H.R., Jiao, Z.X., Li, Q., Liu, S.K., 2019a. Comparative transcriptome analysis reveals molecular basis underlying fast growth of the selectively bred Pacific oyster, *Crassostrea gigas*. Front. Genet. 10, 610.
Zhang, H., Wang, H., Chen, H., Wang, M.Q., Zhou, Z., Qiu, L.M., Song, L.S., 2019b. The transcriptional response of the Pacific oyster *Crassostrea gigas* under simultaneous bacterial and heat stresses. Dev. Comp. Immunol. 94, 1–10.