Combined analysis of mRNA and miRNA reveals the mechanism of pacific white shrimp (*Litopenaeus vannamei*) under acute alkalinity stress

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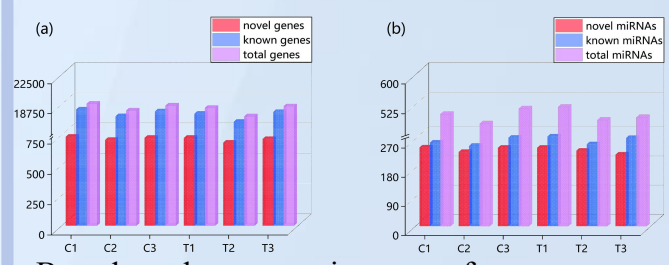
Abstract

The pacific white shrimp is now a more common aquaculture species in saline-alkali waters, while alkalinity stress is considered to be one of the stressors for shrimp. Thus, an understanding of the molecular response to alkalinity stress is critical for advancing the sustainability of culture in pacific white shrimp. In this study, we aimed to explore the response mechanism to acute high-alkaline stress by RNA-seq at low-alkaline (50 mg/L) and high-alkaline (350 mg/L). We detected several genes and miRNAs which were identified as candidate regulators of alkalinity stress, and expression patterns of key genes related to alkalinity stress in pacific white shrimp. Among these genes, the expression levels of most key genes enriched in ion regulation, digestion and immunity were increased, and the expression levels of genes enriched in metabolism were down-regulatedThis research indicated that the homeostatic regulation and digestion changed significantly under acute alkaline stress, and the variations from metabolic and immunity can cope with the osmotic shock of alkalinity stress in pacific white shrimp.

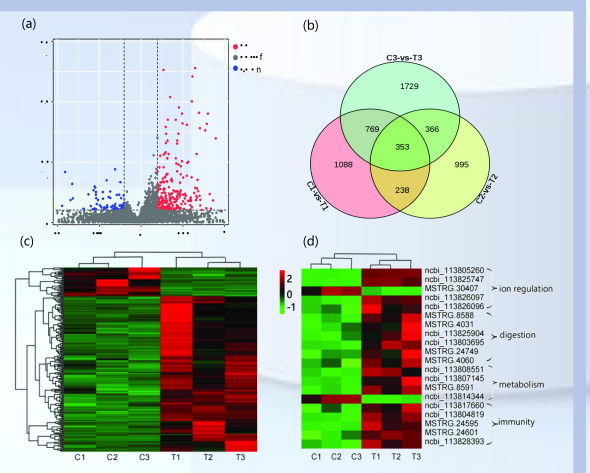
Results

Fig 1



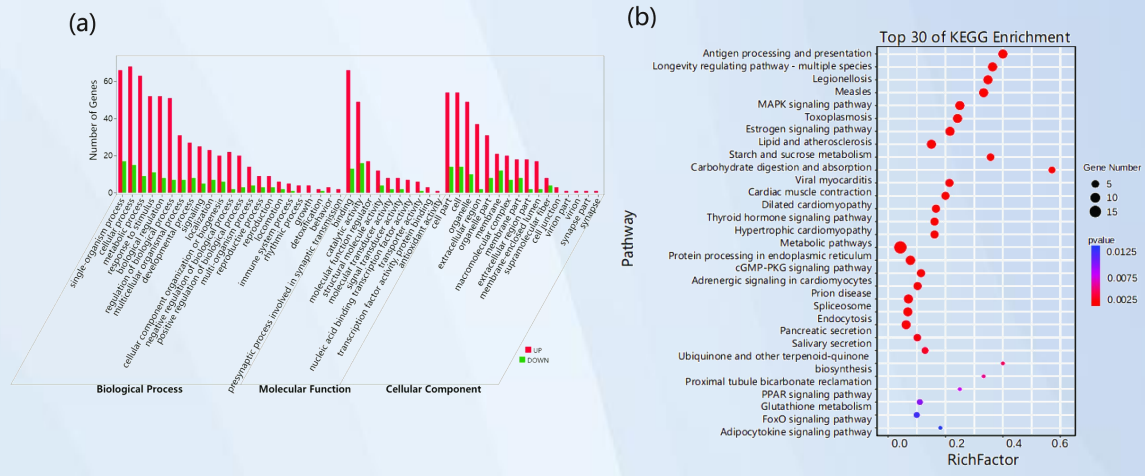
Based on the comparison to reference genome sequence, 772 novel genes, 24,977 known genes and 25,749 total genes were identified in C and T groups, and the detailed comparison information of C1, C2, C3, T1, T2 and T3 were shown in the Fig 1A. A total of 294 novel miRNAs, 335 known miRNAs and 629 total miRNAs were identified in C and T groups, and the detailed comparison information (C1, C2, C3, T1, T2 and T3) in the Fig 1B.

Fig 2



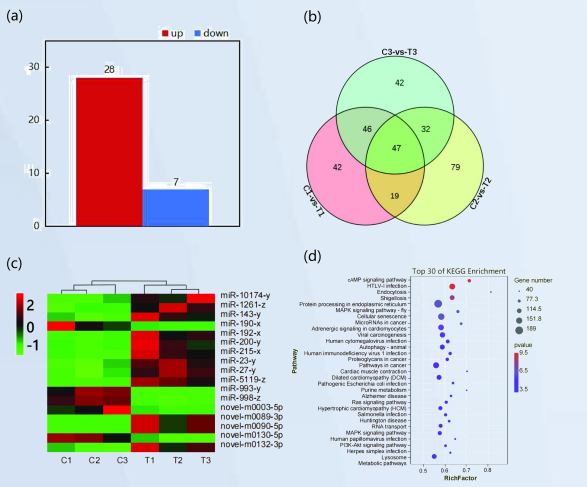
A total of 215 significant DEGs were identified in group T compared with group C, of which 180 DEGs were up-regulated and 35 DEGs were down-regulated. The veen diagram indicated the overall number of differential genes with 353 DEGs were co-expressed. Hierarchical clustering analysis of all DEGs showed high repeatability. Among these DEGs, it was worth noting that many ion regulation, metabolism, digestion and immunity related DEGs were significantly up-regulated, and expression data of their expression and subvariety clustering between C and T groups were respectively shown in Fig 2D.

Fig 3



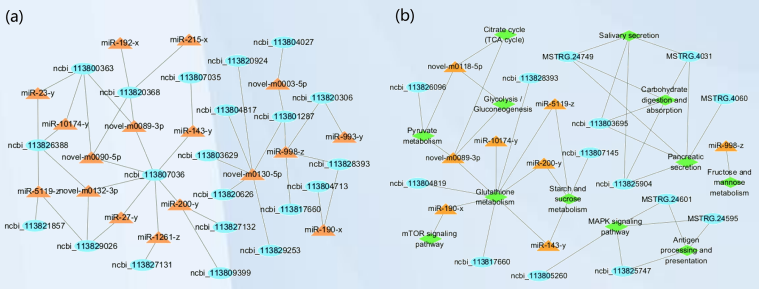
To further investigate the function of DEGs, GO enrichment and KEGG pathway analyses were conducted. Hypergeometric tests were then applied to identify 49 GO terms that were significantly enriched in differential genes compared to background. KEGG pathway analysis identified the top 30 pathways that were significantly different under alkalinity stress. Among these pathways, “Infectious diseases”, “Cardiovascular diseases”, “Circulatory system”, “Immune system”, “Endocrine system” and “Digestive system” were the most significantly enriched pathway subclasses. In addition, a number of important pathways were also significantly enriched, the specific information was as follows. “Proximal tubule bicarbonate reclamation”, “MAPK signaling pathway” and “cGMP—PKG signaling pathway” were related to homeostatic regulation. “Carbohydrate digestion and absorption”, “Salivary secretion” and “Pancreatic secretion” were related to digestion. The metabolism related pathways were “Starch and sucrose metabolism” and “Metabolic pathways”, and the immune-related genes enriched in pathways, including “Antigen processing and presentation” and “Glutathione metabolism”.

Fig 4



By mRNA sequencing data analysis, 35 significant DEMs were identified in T compared with C, of which 28 DEMs were up-regulated and 7 were down-regulated. The GO results showed
that the target genes of C and T were significantly enriched in differential genes with 66 GO erms. Based on three categories, the 6 top terms were cellular process, single-organism process, response to stimulus, cell and cell part, which were generally consistent with the results of GO terms analysis of DEGs. KEGG results showed that among these pathways, the most significantly enriched pathway class was “Human Diseases”, and some important pathways were also significantly enriched, including “Environmental Information Processing”, “Cellular Processes”, “Genetic Information Processing”, “Organismal Systems”, and “Metabolism” subclass. The “MAPK signaling pathway” related to homeostatic regulation; the “Lysosome” related to digestion; and the lipid-related genes were also enriched in the “Metabolic pathways” pathway.

Fig 5



To explore the role of DEMs, we predicted the target genes that might have regulatory relationships with DEGs, and a total of 42 negatively associated miRNA-mRNA pairs were obtained, in which 35 DEMs and 215 DEGs were involved. Enrichment analysis of GO and KEGG were performed to filter the key terms and pathways, which played vital roles in the process of alkalinity stress with target genes of DEMs. We observed significant gene enrichment of GO terms that related to stress response including response to stimulus, biological regulation and metabolic process. Additionally, KEGG enrichment analysis showed that many pathways were significantly enriched including “Glutathione metabolism”, “Notch signaling pathway”, “mTOR signaling pathway” and “Metabolic pathways”. Furthermore, to investigate the potential role of regulatory relationships between these target genes, we used Cytoscape to construct miRNA-mRNA interaction network of selected pathways. miR-200-y, miR-5119-z, miR-998-z, novel-m0089-3p, novel m0090-5p, novel-m0130-5p and novel-m0132-3p were the miRNA targets that regulated more mRNAs. We also constructed gene-pathway interaction network. mRNAs and miRNAs were related to ion regulation, digestion, metabolism and immunity, and they main enriched in the pathways as below “Glutathione metabolism”, “Carbohydrate digestion and absorption”, “Salivary secretion”, “Pancreatic secretion” and “Starch and sucrose metabolism”.

Conclusions

This study investigated the molecular response mechanism of pacific white shrimp under alkalinity stress by RNA-seq. Under acute alkalinity stress, the expression levels of most key alkalinity stress-related genes enriched in ion regulation, digestion and immunity increased, and the expression levels of genes enriched in lipid metabolism were down-regulated. This research indicated that the genes and miRNAs related to homeostatic regulation, digestive, metabolic and immunity have changed significantly under alkalinity stress in pacific white shrimp. The results provide basic data for further analyzing the molecular mechanism under alkalinity stress, and also provide theoretical basis for optimizing the culture technology in pacific white shrimp.