Effect of Low-Temperature Stress On Na^+/K^+-ATPase and Transcriptome Changes in *Oreochromis Niloticus* Gill Tissues

Zhe Li
Southwest university
Luting Wen
Guangxi Institute of Fishery Sciences
Xia Wu
Guangxi Normal University
Junqi Qin
Guangxi Institute of Fishery Sciences
Zhong Chen
Guangxi Institute of Fishery Sciences
Xianhui Pan
Guangxi Institute of Fishery Sciences
Kangqi Zhou
Guangxi Institute of Fishery Sciences
Yin Huang
Guangxi Institute of Fishery Sciences
Qian Deng
Guangxi Institute of Fishery Sciences
Yong Lin
Guangxi Institute of Fishery Sciences
Xuesong Du (627380756@qq.com)
Guangxi Institute of Fishery Sciences

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Abstract

Low temperatures limit the development of *Oreochromis niloticus* (tilapia), and an increase in low-temperature tolerance would increase yields. We studied the responses of tilapia to low temperatures. The fish were labeled CK, AA, BB, and CC based on treatment (25°C, 12°C/1 h, 12°C/24 h, and 12°C/48 h, respectively) with CK being the control group. We examined the transcriptome responses and the Na^+/K^+-ATPase activity of gill tissue in each group. The Na+/K+-ATPase activity varied with the treatment time. Transcriptome sequencing of 12 individuals yielded 585.51 million clean reads, and at least 83.26% of the genes were mapped to the reference genome. Comparative analysis revealed 12,448 genes with significantly differential expression, including 792, 1,827, 1,924 upregulated genes and 992, 3,056, 3,857 genes downregulated for AA, BB, and CC, respectively. Differentially expressed genes (DEGs) were validated using RT-PCR for five genes. Functional annotation analysis of the DEGs identified functions associated with response to low-temperature stress. When tilapia was subjected to low-temperature stress, expression changes occurred in genes associated with cytokine-cytokine receptor interaction, metabolic pathways, cell adhesion molecules, material transport, and immunity. The founding will help understand the effects of low temperature on fish and provide a theoretical basis for the tilapia breeding industry.

Introduction

Ambient temperature restricts the distribution, breeding, and migration of cold water vertebrates (Zhang et al. 2006). Fish are poikilothermic species that are adapted to daily and seasonal temperature fluctuations in their aquatic environments (Zhou et al. 2019). Some fish species have a high ambient tolerance range. These include *Siniperca chuatsi* (0–30°C), *Leiocassis longirostris* (0–38°C), and *Ictalurus punctatus* (0–38°C) (Long 2005). However, low-temperature stress exceeding thermal tolerance capability can be harmful, or even fatal, to fish (Donaldson et al. 2008). Low-temperature stress has limited aquaculture industry development of these fish.

Oreochromis niloticus (tilapia) is an important tropical fish widely cultured in the torrid and subtropical zones (Yang et al. 2015). It is a high-quality species recommended by the United Nations for its fast growth rate, relatively low production cost, and high tolerance to adverse conditions (Zhu 2004). Tilapia was introduced into China in 1978 and is now widely cultivated in Guangdong, Guangxi, and Fujian provinces (Zhou et al. 2019). The tilapia aquaculture industry is constrained by the water temperature. Tilapias can grow between 16°C and 38°C, and optimum growth occurs between 25°C and 28°C (Wohlfarth and Hulata 1983). They are unable to thrive at low temperatures (Cnaani et al. 2000), and tilapia breeding suffers from low winter temperatures. The range of the aquaculture tilapia aquaculture industry would be expanded if tilapia possessed greater resistance to low temperatures.

Transcriptome analysis by RNA-seq can provide a high-throughput tool to discover differentially expressed genes (Wang et al. 2009). It has been used to study the mechanism of low-temperature tolerance in fish, including *Oreochromis aureus* (Nitzan et al. 2019) and *Cyprinus carpio haematopterus*

(Bin et al. 2015). Transcriptome changes can occur in *Oreochromis niloticus* under low-temperature stress based on the transcriptome changes of liver and kidney tissues. However, no transcriptome changes in gill tissues have been reported. Bin et al. (2015) showed that low-temperature stress can cause liver, spleen, and gill tissue damage of tilapia, with lower temperatures causing more serious damage. Evans et al. (2005) showed that fish gills, in addition to functioning as a breathing organ, are also the primary site for osmoregulation, excretion of nitrogenous waste products, and metabolism of hormones and xenobiotics. Chen (2011) showed that *Ctenopharyngodon idellus*, *Aristichthys nobilis*, and *Hypophthalmichthys molitrixcould* could change their gill structure to adapt to low temperatures. This suggested that gill tissues play an important role in resisting low-temperature stress. Therefore, the transcriptional spectrum of gill tissues might provide insight into the adaptation of fish to low-temperature stress.

In this study, we examined the transcriptome responses and the Na^+/K^+-ATPase activity of gill tissue under low-temperature challenge. We used Illumina sequence technology to sequence the RNA, and the sequencing reads were mapped to the *Oreochromis niloticus* reference genome. Subsequently, Gene Ontology (GO) functional enrichment and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis were used for the analysis of differentially expressed genes (DEGs). The objective of this study was to identify the gill response to low-temperature stress in tilapia and to investigate the molecular mechanisms involved in its susceptibility to low-temperature stress.

Materials And Methods

Experiment design and sampling

Forty-eight individuals of *Oreochromis niloticus* (average body weight 46.701 g, average body length 10.369 cm) were collected from the Guangxi Academy of Fishery Sciences in Nanning, China. Forty-eight individuals were randomly divided into four groups (12 individuals in each group), and each group was reared in a separate tank (60 × 50 × 50 cm) for 7 d under laboratory conditions. (The water temperature was maintained at 25±1°C, with the pH between 7.0 and 7.6, and the dissolved oxygen was 5–7 mg/L.) They were fed 3% of their body weight with the same commercial diet daily. For cold treatment, we put ice cubes into the tank to lower the water temperature and to assure that the temperature gradually decreased from 25°C to 12°C in 39 h at a rate of ∼1°C/3 h and was then maintained at 12°C for 48 h. During the experiment, the fish were labeled as CK, AA, BB, and CC corresponding to the temperatures studied (25°C, 12°C/1 h, 12°C/24 h, and 12°C/48 h, respectively) (Fig. 1). CK was the control group. Three individuals of each group were randomly selected, and a total of 12 individuals were anesthetized with tricaine methanesulfonate (MS-222, 100 mg/L) and sacrificed via decapitation for subsequent sampling. Gill tissues of each experimental fish were divided into two parts: one part was placed in the tissue RNA protective solution (RNAsafety^{TM}), whereupon the tissues were stored at −80°C until use, and the other part was stored in a −80°C ultra-low freezer for Na^+/K^+-ATPase activity determination.

Na+/K+-ATPase activity determination

The Na+/K+-ATPase activity was investigated in the crude gill homogenates following previously published methods (McCormick 2011) and (Lin and Randall, 1993), as modified by Nawata et al. (2007). The protein concentrations were measured by an A045-4 BCA protein concentration determination kit (Nanjing Jiancheng Biological Engineering Institute, China), and the activity was expressed as U/mgprot.

RNA Extraction, library construction, and sequencing

Total RNA was extracted using a Trizol reagent kit (Invitrogen, Carlsbad, CA, USA) according to manufacturer protocol. RNA quality was assessed on an Agilent 2100 Bioanalyzer (Agilent Technologies, Palo Alto, CA, USA) and checked using RNase-free agarose gel electrophoresis. After total RNA was extracted, eukaryotic mRNA was enriched by Oligo(dT) beads. Then the enriched mRNA was fragmented into short fragments using fragmentation buffer and reverse transcribed into cDNA with random primers. Second-strand cDNA was synthesized by DNA polymerase I, RNase H, dNTP, and buffer. The cDNA fragments were purified with a QiaQuick PCR extraction kit (Qiagen, V enlo, The Netherlands), end repaired, poly(A) added, and ligated to Illumina sequencing adapters. The ligation products were size selected by agarose gel electrophoresis, PCR amplified, and sequenced using Illumina HiSeq2500 by Gene Denovo Biotechnology Co. (Guangzhou, China).

RNA-seq reads processing and mapping

Reads obtained from the sequencing machines included raw reads containing adapters or low-quality bases, which can affect assembly and analysis. To obtain high-quality clean reads, reads were filtered by fastp (version 0.18.0) (Chen et al. 2018). The parameters were as follows: removing reads containing adapters; removing reads containing more than 10% of unknown nucleotides (N); removing low-quality reads containing more than 50% of low-quality (Q-value ≤ 20) bases. To obtain clean reads without Ribosome RNA (rRNA), the Short reads alignment tool Bowtie2 (version 2.2.8) was used for mapping reads to ribosome RNA (rRNA) database (Langmead and Salzberg 2012). The rRNA mapped reads were then removed. The remaining clean reads were further used in assembly and gene abundance calculation. An index of the reference genome was then built, and paired-end clean reads were mapped to the reference tilapia genome using HISAT2. 2.4 with “rna-strandness RF” and other parameters set as default values (Kim et al. 2015).

Analysis of differentially expressed genes

The mapped reads of each sample were assembled using StringTie v1.3.1 in a reference-based approach. For each transcription region, a FPKM (fragments per kilobase of transcript per million mapped reads) value was calculated to quantify its expression abundance and variations, using StringTie software (Pertea et al. 2016). RNAs differential expression analysis was performed by DESeq2 software between two different groups (and by edgeR between two samples) (Robinson et al. 2010; Love et al. 2014). The transcripts with a false discovery rate (FDR) below 0.05 and absolute fold change ≥ 1 were

considered to be differentially expressed genes. DEGs were further annotated by GO and KEGG pathway analyses. STEM (Short Time-series Expression Miner) software (v.1.3.11) was applied to compare the DEGs, which were co-expressed DEGs in three datasets (Ernst and Bar-Joseph 2006).

Real-time RT-PCR confirmation of Illumina sequencing data

To validate the reliability of the transcriptome data, five genes were selected, and their mRNA levels were measured by real-time PCR (RT-PCR). Gill tissues from 12 individuals (three biological replicate sample pools) of four groups were sampled and were used in the RT-PCR. Total RNA was extracted using a Trizol reagent kit (Invitrogen, Carlsbad, CA, USA) according to manufacturer protocol. RT-PCR was conducted with ABI Step OnePlus. Nicotinamide adenine dinucleotide phosphate (NADPH) was used as the normalization control to calculate the relative expression levels. The melting curve was analyzed to confirm the target specificity. All samples were performed in triplicate, and relative gene expression levels were calculated using the $2^{-\triangle\triangle CT}$ method (Livak and Schmittgen 2001).

Result

Gill Na+/K+-ATPase activity

The Na^+/K^+-ATPase activity of gill tissue in each treatment group is illustrated in Fig. 2. In AA, the Na+/K+-ATPase activity was significantly higher than in the CK ($P<0.01$). At the time of low-temperature stress prolongation, the Na^+/K^+-ATPase activity of BB and CC was significantly lower than AA ($P<0.05$). The differences between CC and CK were significant ($P<0.05$), while the Na+/K+-ATPase activity of BB was similar to CK ($P>0.05$).

Transcriptome profiles and annotation

A total of 593.19 million raw reads were obtained by sequencing 12 cDNA libraries. After removal of adaptor and primer, ploy-N-containing reads, Ribosome RNA (rRNA), and low-quality reads, about 585.51 (98.70%) million clean reads were obtained (Table 1). The uniquely mapped percentages of these transcripts ranged from 83.26% to 84.62%, while the percentage of multiple mapped transcripts ranged from 2.30% to 2.80%. The average Q20 and average Q30 of AA reached 97.98% and 97.92%, the average GC content of the experimental group was 45.27%, and the average Q20 and Q30 were 95.74% and 89.93%. The error rate was 0.03%, indicating that the clean reads can be used for subsequent biological information analysis.

Differentially expressed genes

During the RNA mapping, at least 83.26% of the clean reads were aligned to the tilapia reference genome. Statistical analysis performed with the DESeq2 package identified the differentially expressed genes between the CK and the cold treatments (AA, BB, CC). A total of 12,448 DEGs were identified between CK and AA, BB, and CC (Fig. 3). Compared to CK, there were 1,784 DEGs, of which 792 were

upregulated and 992 were downregulated in AA (Fig 3A). In BB, 4,883 DEGs were identified, of which 1,827 were upregulated and 3,056 were downregulated (Fig 3B). In CC, 5,781 DEGs were identified, of which 1,924 DEGs were upregulated and 3,857 were downregulated (Fig 3C). The response to low temperature was strong in terms of the total number of DEGs and also in the degree of transcriptional changes. A total of 478 DEGs in AA (212 over-expressed and 266 under-expressed), 1,642 DEGs in BB (511 over-expressed and 1,131 under-expressed), and 2,310 DEGs in CC (554 over-expressed and 1,756 under-expressed) showed large FCs ($|\log2FC| \geq 2$). The global gene expression profiles of tilapia in AA, BB, and CC showed large differences when compared to CK under low-temperature stress conditions. In addition, the number of DEGs increased with treatment time. Through sequence alignment, many functional genes were recognized, such as *cell division cycle 42* (*cdc42*), *aquaporin families* (*aqps*), *FK506-binding protein 5* (*fkbp5*), *heat shock protein family A (Hsp70) member 4* (*hspa4a*), and *toll-like receptor 5* (*tlr5*). These genes may increase our understanding of the response of tilapia to low-temperature stress.

Gene Ontology (GO) and KEGG pathway enrichment

GO enrichment and KEGG pathway enrichment analysis were performed using the corresponding annotations of genes to understand the functional relevance of the DEGs. Compared to CK, 49,759 DEGs could be assigned by GO classification. Of these, there were 6,414, 19,656, and 23,689 DEGs in the CK/AA, CK/BB, and CK/CC comparisons, respectively (Fig. 4). Compared to CK, the top five significant annotated GO enrichments in the cold treatment groups were all binding (GO:0005488), cellular process (GO:0009987), single-organism process (GO:0044699), metabolic process (GO:0008152), and catalytic activity (GO:0003824). The genes enriched to these five pathways accounted for 42.33%, 40.39%, and 40.57% of the total genes in each group. The classified genes (groups CK, AA, BB, and CC) produced 599 different pathways, including organismal systems, environmental information processing, human diseases, metabolism, and cellular processes. In the AA/CK comparison, 564 DEGs were assigned to 203 pathways (Fig. 5). In the CK/BB comparison, 1,023 DEGs were assigned to 227 pathways. Compared to CK, 1,234 DEGs were assigned to 216 pathways in CC. Compared to CK, the top three KEGG pathways with the largest number of enriched genes in AA, BB, and CC were all cytokine-cytokine receptor interaction (ko04060), metabolic pathways (ko01100), and cell adhesion molecules (CAMs) (ko04514). According to the KEGG function annotations, a total of 55 significantly enriched pathways (P-value < 0.05) were identified in the CK/AA, CK/BB, and CK/CC comparisons.

Trend analysis of differentially expressed genes

A total of 1,305 DEGs were performed by STEM to study the transcriptome changes of gills during low-temperature stress. A total of 1,305 were classified into eight profiles (Fig. 6), and among them, two significant profiles were identified (P<0.01). These included one upregulated profile (profile covering 445 DEGs) and one downregulated profile (profile covering 761 DEGs).

Validation of RNA-Seq Profiles by RT-PCR

To validate the gene profiles from RNA-seq, the expression of five genes was measured by real-time PCR analyses (Fig. 7). The melting-curve analysis showed a single product for all selected genes. The overall consistency of expression from RNA-seq and RT-PCR for the majority of genes revealed the sequence assembly, and the expression analysis of genes provided transcriptome information.

Discussion

Low-temperature stress can reduce the concentration of sodium ions and chloride ions in the plasma and reduce the osmotic pressure of *Tilapia mossambica* (Allanson et al. 2006). Tilapia can cope with this by adjusting the mRNA expression of *aqps* and Na+/K+-ATPase activity to sustain intracellular homeostasis and regulation of cell osmotic pressure (McCormick 1995; Gonen and Walz 2006). Na+/K+-ATPase is a universal membrane-bound enzyme that can actively regulate the transmembrane movement of Na^+ and K^+ (Lin et al. 2003). In this study, the Na+/K+-ATPase activity increased at the second-time point after low-temperature stress, then decreased to a minimum at the fourth-time point. During low-temperature treatment, at a water temperature of 12°C, tilapia stop eating, swimming is reduced, and their breathing rate is minimal (Bin et al. 2015). At 12°C, tilapia may increase their rate of respiration to adapt to the low-temperature stress (Haque and Roy 2013). Low-temperature stress can lead to enhanced metabolism of fish (Xu et al. 2012). That may explain why the Na+/K+-ATPase activity increased at the second-time point after low-temperature stress. A similar result was described by Xu et al. (2012). However, the Na+/K+-ATPase activity was decreased gradually with the prolongation of low-temperature stress, and the Na+/K+-ATPase activity in BB was similar to CK. This may be because the cell osmotic pressure in the gills of tilapia is rebalanced by active adjustment, and the Na+/K+-ATPase activity decreases and stabilizes. A study of *Portunus trituberculatus* divided the osmotic regulation of Na+/K+-ATPase activity into three phases: passive stress phase, positive regulation phase, and adaptive phase (Jiang 2012). This suggests that tilapia have an active adaptation period during low-temperature stress. But the Na+/K+-ATPase activity decreased significantly under the following low-temperature stress. Similarly, a study on *Oreochromis mossambicus* confirmed that the activity of Na+/K+-ATPase was reduced under long-term low-temperature stress. Bin et al. (2015) also showed that the gill fragments are severely broken, and the capillaries are severely congested when tilapia live in 12°C water for long time periods. The reason why tilapia did not exhibit a passive stress phase may be that the second sampling time was too late or that tilapia completely lacks a passive stress phase. Therefore, during the 12°C stress, we divided the Na+/K+-ATPase activity of tilapia gills into three phases: positive regulation phase, adaptive phase, and damaged phase.

Aquaporins (aqps) are cell membrane channel proteins that transport water molecules selectively and efficiently (Gonen and Walz 2006). Aqps are important for maintaining homeostasis of the intracellular and extracellular environment (Gonen and Walz 2006). Aqps are also linked to cold resistance in fish (Zhu et al. 2014; Pang et al. 2015; Bin et al. 2021). In the present study, some genes related to aqps were identified, and three genes (*aquaporin-1* (*aqp1a.1*), *aquaporin-1* (*aqp1a.2*), *aquaporin-3* (*aqp3a*)) were classified into a downregulated profile in trend analysis. This suggests that the mRNA expressions of

aqps were related to tilapia resistance to low temperature. Zhu et al. also showed that, compared with non-low-temperature-resistant tilapia, the expression of *aqp1* was downregulated in low-temperature-resistant tilapia (Zhu et al. 2014). Li et al. ((Li et al. 2021) also showed that the expression of *aquaporin-1* (*aqp1*) of low-temperature-resistant *Larimichthys crocea* was more downregulated than non-low-temperature-resistant *Larimichthys crocea*. This study provides evidence that tilapia may reduce the transport of water molecules by downregulating the expression of aqps, maintaining homeostasis, and resisting damage to cells caused by low temperature. In addition, we found that the genes in ABC transporters were upregulated during the low-temperature stress, and the trend analysis also showed that three genes (A*TP-binding cassette, sub-family B (MDR/TAP), member 6a* (*abcb6a*), *ATP-binding cassette, sub-family C (CFTR/MRP), member 6a* (*abcc6a*), *ATP-binding cassette sub-family A member 1 isoform X4* (*abca1*)) related to ABC transporters were classified into the upregulated profile (profile 7). This also indicates that tilapia can resist the damage caused by low temperature by actively transporting and adjusting the osmotic pressure.

Water temperature is the main environmental factor that changes the immune response of fish (Xu et al. 2012). A change in the water temperature could affect fish immunity by decreasing the disease resistance capability (Velmurugan et al. 2019). In our experiment, many genes related to immune response were downregulated in the DEGs analysis, including *tlr5*, *toll-like receptor 8* (*tlr8*), *toll-like receptor 9* (*tlr9*), *toll-like receptor 22* (*tlr22*), and *fkbp5*. These genes can affect the innate immunity that TLRs mediate, and cellular immunity. Trend analysis showed 27 genes related to the immune system were classified into the downregulated profile (profile 0). This indicates that when tilapia is cultured at low temperature, it would have an increased risk of pathogen infection. The pro-immune protein, *fkbp5*, plays an important role in the immune system and immune regulation of the body (Storer et al. 2011). In this study, during low-temperature stress, the expression of *fkbp5* was upregulated. Compared to CK, the mRNA expression of *fkbp5* was upregulated by 3.951-fold, 5.293-fold, and 5.412-fold in AA, BB, and CC, respectively. The in vitro assay results showed that *fkbp5* could inhibit calcineurin phosphatase activity when complexed with *fk520*. This proved that an immunosuppressant inhibits the Ca^{2+} ion signal pathway by binding to *fkbp5*, and then inhibits the activation process of T cells, thus exerting its immunosuppressive effect (Baughman et al. 1995). High expression of *fkbp* can also inhibit the immune response of *Locusta migratoria manilensis* and accelerate the infection of *Metarhizium anisopliae* (Tian et al. 2021). Th{Citation}is suggests that low-temperature stress promotes the expression of *fkbp5*, which inhibits the activation process of T cells and leads to a decrease in the immune capacity of tilapia. We found that low-temperature stress reduced the mRNA expression of immune-related genes.

This study identified the mRNA in gill tissue of tilapia under low-temperature stress. Some immune-related genes and transport-related genes were significantly different compared to the control group. The gene *fkbp5* significantly affects the proliferation of tilapia T cells, which could help explain why low-temperature stress affects tilapia resistance to pathogenic bacteria.

Declarations

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Conflicts of interest Authors declare that they have no conflict of interest.

Availability of data and material Raw sequencing data has been uploaded to NCBI SRA (SRP334133).

Code availability Not applicable.

Authors' contributions Zhe Li, Luting Wen, and Xia Wu analyzes data and wrote the paper. Junqi Qin, Zhong Chen, Xianhui Pan, Kangqi Zhou, Yin Huang, Qian Deng, Zhe Li, Luting Wen, Xia Wu, and Xuesong Du performed the experiments, Yong Lin and Xuesong Du designed the experiments and responsible for experimental guidance.

Additional declarations for articles in life science journals that report the results of studies involving humans and/or animals Not applicable.

Ethics approval All experiments were performed in compliance with the animal management regulations of the Animal welfare and Ethical Committee of Guangxi Academy of Fisheries Science (Nanning, RP China) (GACUC number 201703021), Southwest University (Chongqing, RP China) (Approval ID: 20160922), and Guangxi Normal University (Guilin, RP China) (No. 20150325-XC). All handling of animals in the present study was as according to standard animal care and use practices.

Consent to participate All the authors declare that they are agree with the experiment.

Consent for publication The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper. The authors declare that they have no competing financial interests or personal relationships which may be considered as potential competing interests.

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Tables

Table 1 The raw, total clean, total mapped, unique mapped and multiple mapped reads obtained by RNA-seq analysis of tilapia in different groups.

Index	CK	AA	BB	CC
Raw reads	55,034,562	44,578,016	48,591,017	49,527,998
Total clean reads	54,271,007	44,042,585	48,188,363	48,706,989
Total mapped reads	46,650,533	37,880,005	41,924,620	42,263,918
Uniquely mapped	45,295,323	36,775,656	40,686,560	41,096,842
Multiple mapped	1,355,210	1,104,349	1,238,060	1,167,076

Figures

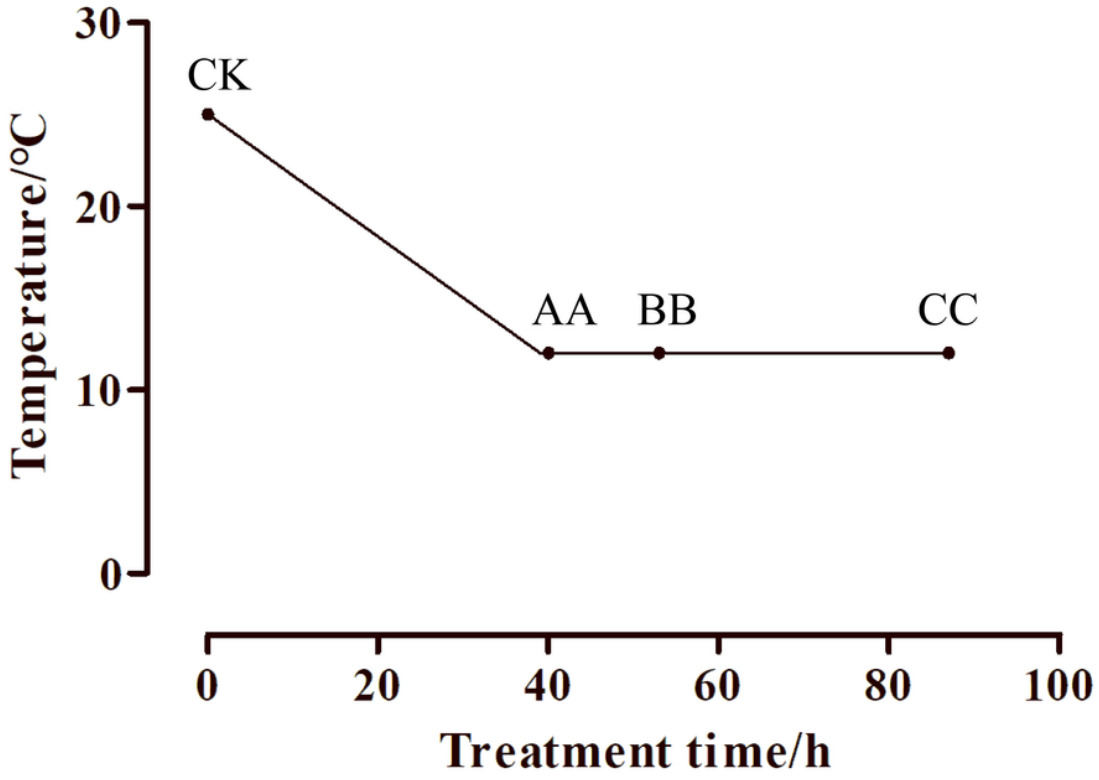

Fig .1The figure shows sampling times of four groups, CK, 25°C, AA, 12°C/1h; BB, 12°C/24h, CC, 12°C/48h.

Figure 1

See image above for figure legend

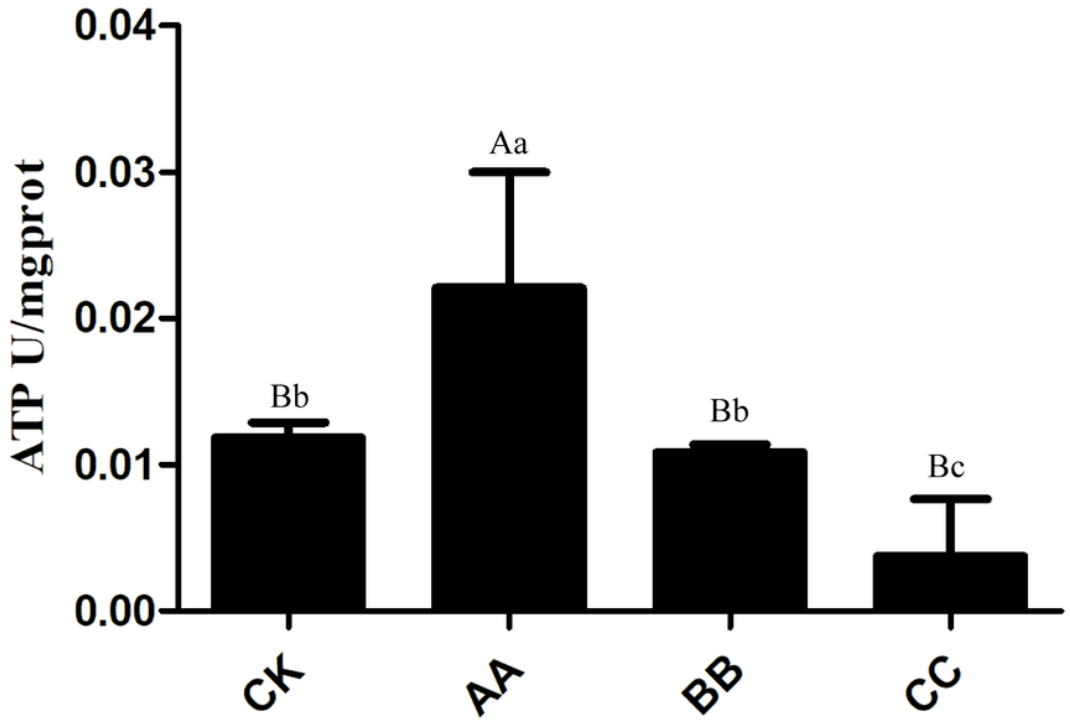

Fig. 2 Gill Na/K-ATPase activity (U/mgprot) of tilapia under different group (CK, AA, BB, CC was cultured in 25°C, 12°C/1 h, 12 °C/24 h, and 12 °C/48 h, respectively.). Values are mean (± SD), and different capital letters and lowercase letters above each bar indicate very significantly different($P<0.01$) and significant differences($P<0.05$) among treatments, respectively.

Figure 2

See image above for figure legend

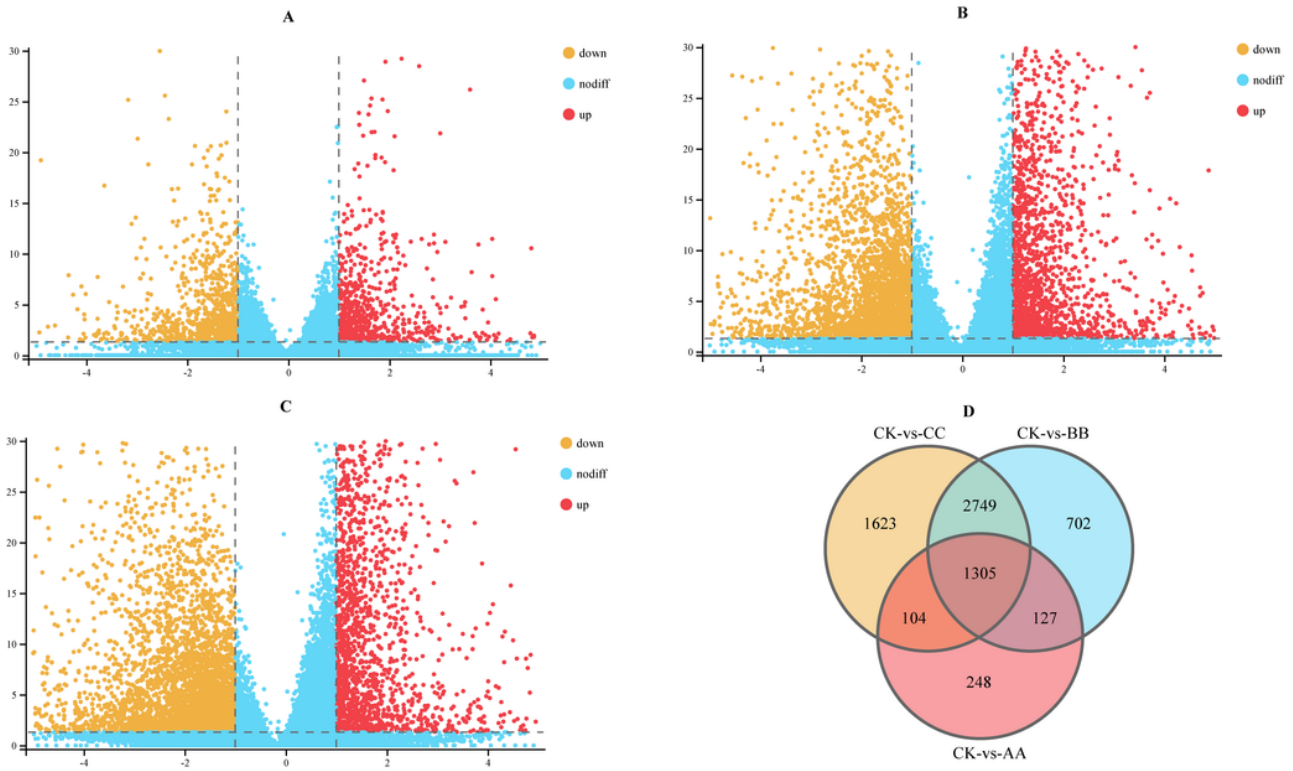

Fig. 3 The Volcano Plot and Venn diagrams of differently expressed genes. A, B, and C were the Volcano Plot of differently expressed genes distribution trends of CK vs AA, CK vs BB, and CK vs CC, respectively. Its X- axis is log2(FC), and the Y- axis is P-value. Each dot represents one gene. Yellow and red dots represent differentially expressed genes (DEGs). Blue dots represent non-differentially expressed genes. D was the Venn diagrams that showed the overlap of DEGs between different time points comparison CK vs AA , CK vs BB, and CK vs CC.

Figure 3

See image above for figure legend

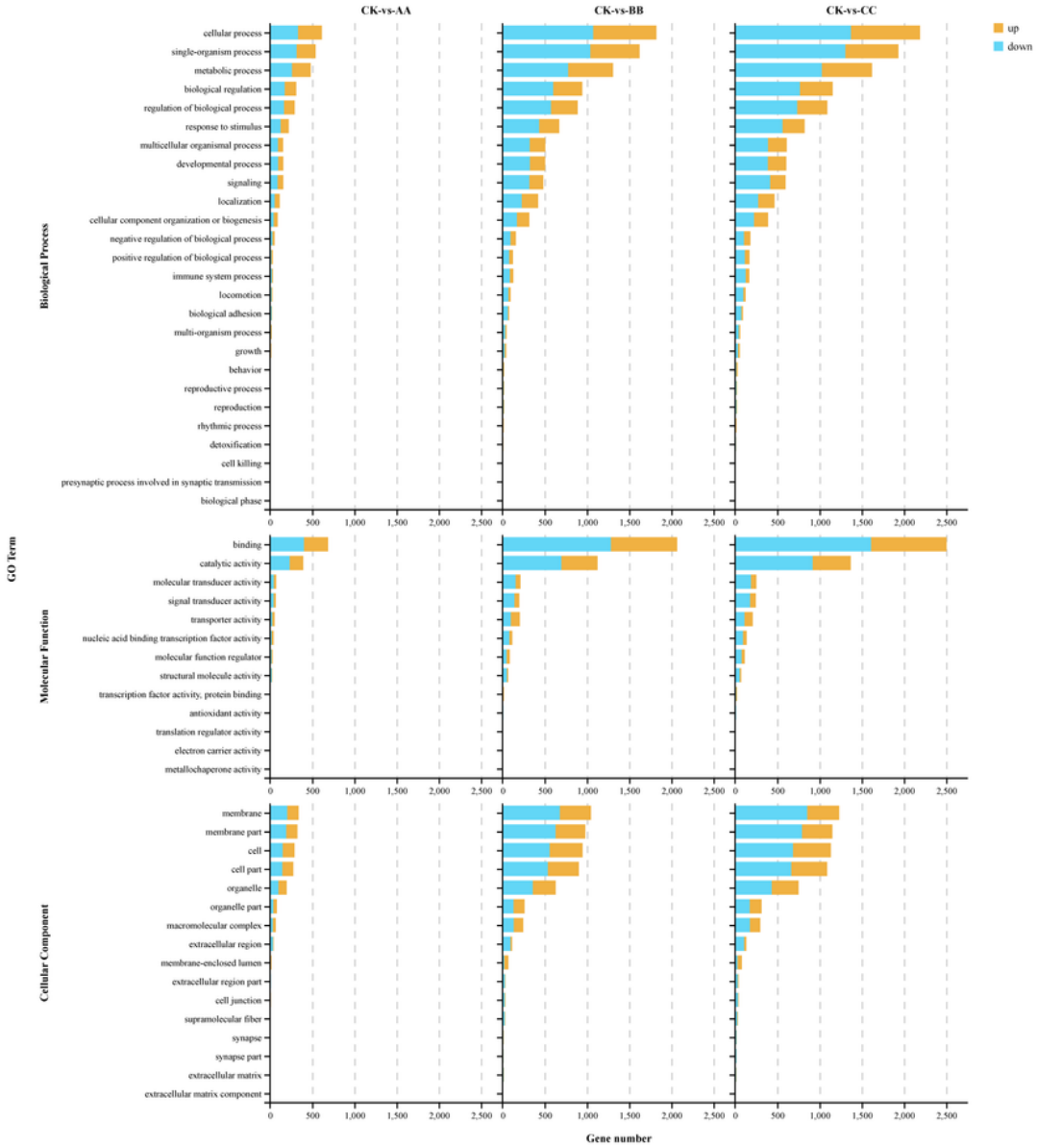

Fig. 4 Gene Ontology functional classification of DEGs. The expression level of AA, BB, CC was compared with control group (CK) and the numbers of significant putative DEGs (either up or down) were showed in x-axis; the Gene Ontology functional classification of putative DEGs was showed in the Y-axis. Up-regulated and down-regulated genes were represented by yellow and blue color. CK, 25°C; AA, 12°C/1h; BB, 12°C/24h, CC, 12°C/48h

Figure 4

See image above for figure legend

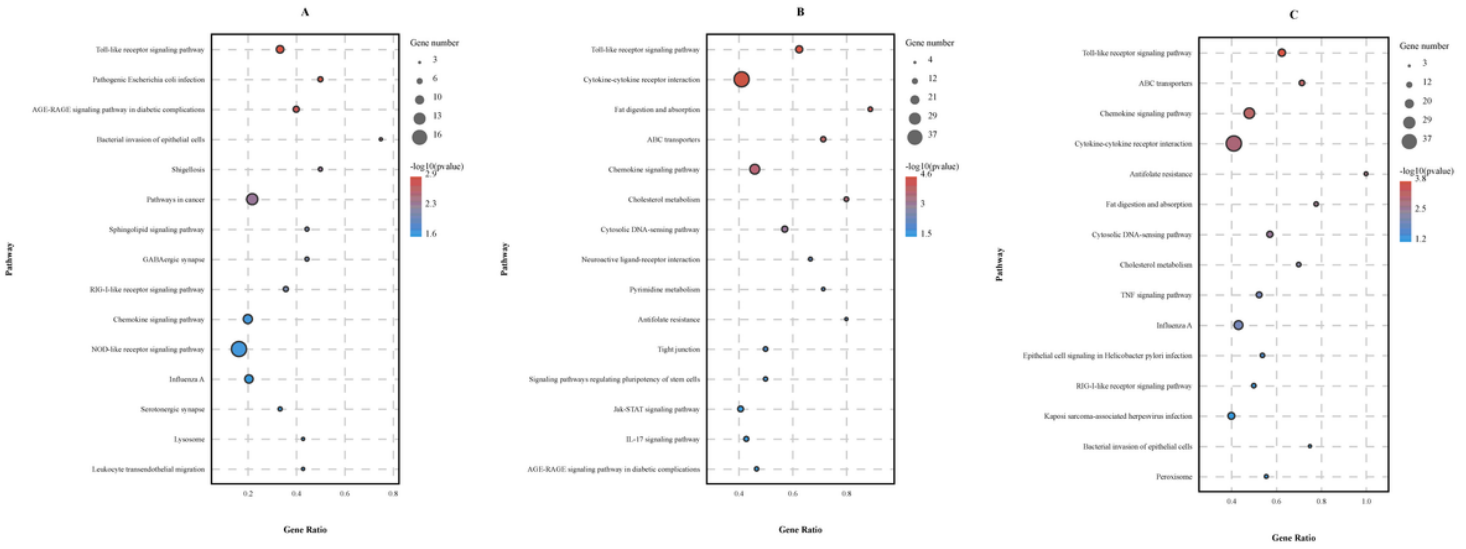

Fig. 5 The KEGG enrichment analysis scatter plot representing pathways of significant DEGs. A, B, and C were the KEGG enrichment analysis scatter plot representing pathways of significant DEGs in response to low-temperature in AA, BB, and CC group, respectively.

Figure 5

See image above for figure legend

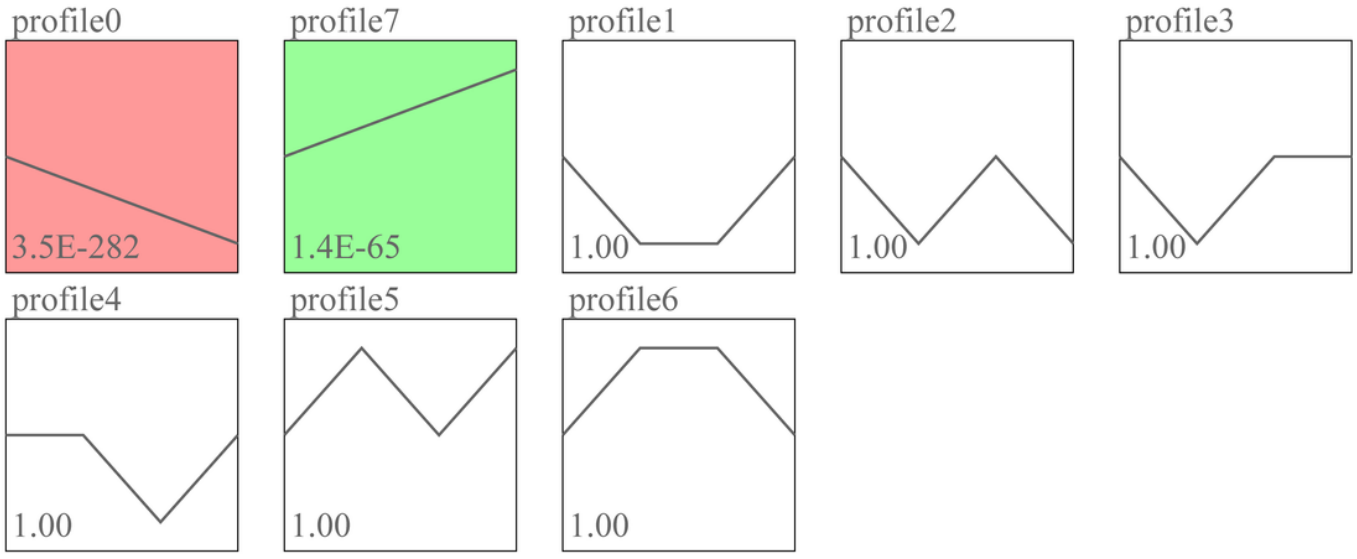

Fig .6 Trend analysis of differentially expressed genes (DEGs) of tilapia subjected to low temperature stress for different time

Figure 6

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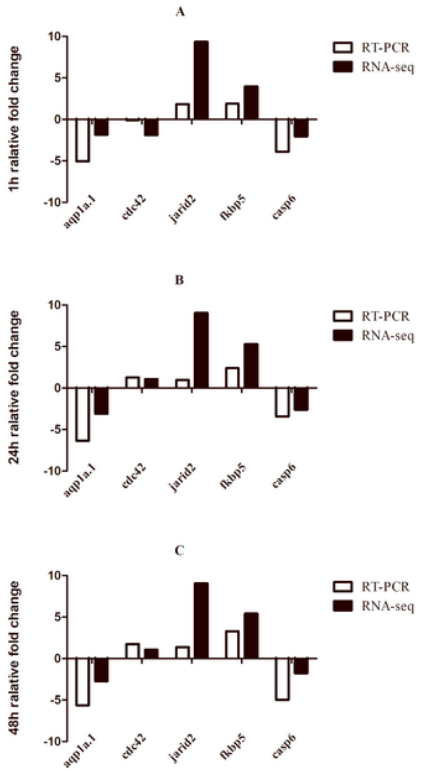

Fig. 7 Comparison of gene expression patterns obtained using comparative transcriptome analysis and RT-PCR. The transcript expression levels of the selected genes were each normalized to that of the NADPH gene. Orange color indicates the value of RNA-seq, blue color indicates the value of RT-PCR. And A, B, C indicate the different treatment time. A, 12°C/1h; B, 12°C/24h, C, 12°C/48h.

Figure 7

See image above for figure legend