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Transcriptomic Analysis Reveals Differential Gene Expression in Himalayan Snow Trout (Schizothorax richardsonii) Challenged with Aeromonas hydrophila

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Abstract

The snow trout (Schizothorax richardsonii), native to the high-altitude Himalayan region, thrives across a broad thermal range (0-27°C). It serves as an excellent model species for investigating the responses of cold-water fish to both abiotic and biotic stressors. In this study, we conducted a comparative analysis of liver transcriptomes from Schizothorax richardsonii challenged with Aeromonas hydrophilla (Ah+) and those mock-challenged (Ar-). The species' transcriptome database was established through RNA sequencing (2×100 bp paired end) using the Illumina 2000 sequencing platform.

Among the 50,453 unigenes assembled de novo, 24,464 were annotated, including 82 associated with immune response genes. We identified 265 differentially expressed unigenes (189 upregulated and 75 downregulated) in the Ah+ group compared to the Ah- group. The majority of these genes involved in immune regulation during bacterial pathogenesis were associated with extracellular regions, membrane integrity, ion binding, signal transduction, antigen processing and presentation, and MHC class I protein complexes. Notably, 15 unigenes associated with immune response and 9 related to signal transduction gene ontology terms showed significant dysregulation in the bacterial-challenged group. Four immune genes—Interleukin 1β1, prostaglandin-endoperoxide synthase 2a & 2b, and ankyrin repeat domain—exhibited high activity against A. hydrophilla. This study offers initial insights into potential molecular changes occurring in this model fish species during pathogenic infestations.

Keywords: Snow trout, Schizothorax richardsonii, Aeromonas hydrophilla, prostaglandin-endoperoxide.

Introduction

The snow trout (Schizothorax richardsonii) is a native Himalayan fish found in various rivers and rivulets across states such as Jammu & Kashmir, Himachal Pradesh, Uttarakhand, Sikkim, and Arunachal Pradesh in India. Known for its adaptation to different ecological niches, this rheophilic and demersal cyprinid species thrives within a temperature range of 0-27°C, spanning from mid to high altitudes in the Himalayas (Barat et al., 2012; Kamalam et al., 2019). As an essential part of the regional ecosystem and a potential candidate for aquaculture, understanding the species' adaptation mechanisms is crucial for its successful management in captive conditions [1].

Despite its significance, limited research exists on the adaptive responses of snow trout to various biotic and abiotic stresses. Previous studies by Barat et al. (Barat, Sahoo, et al., 2016) and Kamalam et al. (Kamalam et al., 2019) shed light on the species' thermal adaptive mechanisms and physiological plasticity, respectively. However, bacterial diseases pose significant challenges to captive aquaculture, emphasizing the need to investigate the molecular response of snow trout to infections caused by Aeromonas hydrophilla [2-5].

To address this gap, our study employs deep RNA sequencing (RNA-Seq), a high-throughput transcriptomic approach, to elucidate the genetic and molecular pathways involved in snow trout's response to aeromoniasis. RNA-seq technology offers several advantages, including the ability to detect novel molecules and pathways without prior gene information, making it particularly suitable for studying non-model organisms (Wang et al., 2009; Qian et al., 2014) [6].

Aeromonad septicemia primarily affects liver and kidney tissues and manifests through diverse pathological symptoms such as dermal ulceration, fin hemorrhages, and necrosis of visceral organs (Sun et al., 2014; Yardimci & Aydin, 2011). A. hydrophila, a common freshwater fish pathogen, has been implicated in disease outbreaks in various fish species, including Clarias gariepinus, Labeo rohita, Sparus aurata, Magalobarma amblycephala, and Tor pititora (Angka et al., 1995; Sahu et al., 2007; Reyes-Becerril et al., 2011; Tran et al., 2015; Kumar et al., 2017). Furthermore, thermally stressed fish are particularly susceptible to Aeromonas infections, resulting in high mortality rates (Noga, 2010; Ortega et al., 1995).

Understanding the defense mechanisms of fish against A. hydrophila is crucial for disease prevention and control (Peatman & Liu, 2007). Therefore, our study aims to compare the liver transcriptomes of A. hydrophilla-challenged (Ah+) and mock-challenged (Ar-) S. richardsonii, providing valuable insights into the molecular responses of snow trout to bacterial infections.

Materials and Methods

Fish Collection and Acclimatization

Forty live specimens of snow trout (Schizothorax richardsonii) were captured using cast nets in the tributary of Kosi River near Ratighat (29° 27.48′ N; 79° 28.81′ E) situated in the Kumaon hills of Uttarakhand, India. Taxonomic identification followed the criteria outlined by Talwar and Jhingran (1991). The collected specimens were acclimatized in aerated water at ambient temperature (10 ± 2 °C) for one month before the experiment. They were fed twice daily with a formulated diet based on 5% of their body weight. The experimental tanks maintained constant aeration and water flow, with regular monitoring of water temperature and physicochemical parameters using a multi-parameter auto-analyzer (Hach®, Colorado, US). All procedures were conducted in compliance with the animal welfare act and approved by the corresponding author's institution [7-9].

Experimental Design and Sampling

Aeromonas hydrophilla (MTCC 646) was cultured in LB medium and suspended in PBS to 1 x 106 CFU/ml. The median lethal dose (LD50) was determined using six fishes per group, with bacterial concentrations of 1×104, 105,106, 107 CFU/mL for 24 hours in a separate experiment. Following a one-week acclimation period, the fishes were randomly distributed into two 1000 L FRP tanks, with five fishes (100–150 gm) per tank. The water temperature was continuously monitored, and the experimental group received an injection of 1×105 CFU of A. hydrophilla (Ah+), while the control group received PBS (Ah-). Three fishes from each tank were sampled at 6 hours post-challenge after anesthesia with an overdose of tricaine methanesulfonate (400 mg/L) (Sigma, St. Louis, USA). Liver tissue was collected from each fish for transcriptome profiling.

NA Isolation, Library Preparation, and De Novo Assembly

Total RNA was extracted from 50–100 mg of liver tissue using the PureLink® RNA Mini Kit (Life Technologies, Carlsbad, USA) following the manufacturer's protocol. Genomic DNA contamination was removed by DNase treatment at 30°C for 30 minutes. RNA concentration and purity were assessed using NanoDrop® 2000 (Thermo Fisher Scientific, Wilmington, USA), while integrity was verified through agarose gel electrophoresis and 2100 Bioanalyzer (Agilent Technologies, Santa Clara, USA) with an acceptable RNA integrity value (RIN) of ≥ 8. RNA samples from each group (Ah+ and Ah-) collected at 6 hours were pooled (three biological replicates) in equal concentrations for library preparation [10-13].

Two paired-end RNA-Seq libraries were prepared using the TruSeq® RNA sample preparation kit (Illumina, San Diego, USA) following the manufacturer's instructions and sequenced on Hi-Seq 2000 (Illumina, San Diego, USA) to generate 2×100 bp sequencing reads at SciGenom Laboratories (SciGenom, Kochi, IND). The fastq files were trimmed and assembled using SOAPdenovo2 (Luo et al., 2012) with default parameters. Trimmed reads were aligned to the assembled transcriptome using the Bowtie program (Langmead et al., 2009) with up to 3-mismatches allowed in the seed region. Differential gene expression analysis was performed using the DESeq program (Anders & Huber, 2010) with a minimum cut-off value for differentially expressed genes of ≥5 fold at p=0.01. Expression levels were calculated as reads per kilobase per million mapped reads (RPKM) for DeSeq analysis. Gene ontology (GO) annotations and Kyoto Encyclopedia of Genes and Genomes (KEGG) assignment were performed using Blast2Go and BLASTx against the KEGG database, respectively.

Results and Discussion

Sequence Analysis and Annotation

Sequencing of liver transcriptomes from Aeromonas hydrophilla-challenged (Ah+) and mock-challenged (Ah-) Schizothorax richardsonii specimens yielded 65,428,283 and 62,387,440 bp, respectively, using Illumina HiSeq 2000 with 2×100 bp paired-end reads. Following data trimming, 62,378,245 and 57,820,543 reads were retained for analysis in Ah- and Ah+ samples, respectively. The de novo assembly produced 50,453 unigenes, with 24,464 (48.49%) annotated via BLASTx and 24,237 (99%) having ≤1e-5 cut-

off values. Uniprot annotation was obtained for 14,291 unigenes, with the majority (80.28%) showing similarity to Danio rerio. Gene ontology (GO) analysis identified 82 unigenes associated with immune responses, including chemokines, MHC class I & II antigens, interleukins, toll-like receptors, etc. Furthermore, 377 unigenes were assigned to the "proteolysis" GO term, and 49 to the apoptotic process [10-14].

Differential Gene Expression Analysis

Differential gene expression analysis revealed 265 differentially expressed unigenes (189 upregulated, 75 down-regulated) in Ah+ compared to Ah-. Notably, 96 upregulated and 21 downregulated genes were assigned GO terms. Among the upregulated genes, five were localized in extracellular regions, while eight were involved in immune responses, including interleukin 17c, tissue inhibitor of metalloproteinase 2b, chemokine CXC-C1c, granulocyte colony-stimulating factor 3, and Thrombospondin-1b. Additionally, several immune-related genes such as prostaglandin-endoperoxide synthase 2a and 2b, chemokine C-X-C motif, immunity-related GTPase family, and complement component showed significant upregulation. Conversely, downregulated genes included MHC class I antigens, immunoglobulin heavy chain, integrin, among others [15].

KEGG Pathways of DEGs

Of the 189 differentially expressed genes, 84 were assigned to KEGG IDs, with 58 genes classified into 132 KEGG pathways. Notably, genes involved in signal transduction, such as Ras signaling, MAPK signaling, NF-Kappa signaling, and TNF signaling pathways, were upregulated. The TNF signaling pathway, for instance, featured upregulated genes like interleukin 1β1, B-cell lymphoma 3 protein homolog, and Prostaglandin endoperoxide synthase 2a. Similarly, immune system-related pathways showed upregulation, with interleukin 1β1 playing a prominent role across multiple pathways.

Conclusion

The snow trout, a member of the Schizothoracinae subfamily within the Cyprinidae family, thrives in extreme cold and pathogen-prone environments, making it a valuable taxon for studying adaptive mechanisms in fish. While this study did not provide definitive conclusions regarding the mechanisms of snow trout response to Aeromonas hydrophila infection, it shed light on several potential mechanisms within the fish's immune response. Cell surface receptors, such as toll-like receptors and c-type lectin receptors, emerge as potential key players in the snow trout's immune response. Additionally, cytokines like IL1β1, B-cell lymphoma 3 protein homolog, and Prostaglandin-endoperoxide synthase 2b, along with caspases, may regulate the balance between humoral and cell-based immunity and orchestrate immune function during bacterial infection. Despite these insights, numerous unanswered questions remain regarding the snow trout's immune response to bacterial infection. Functional studies are necessary to elucidate the roles of specific effectors and unravel the intricate mechanisms underlying pathogenesis in snow trout. Further research in this area will contribute to a deeper understanding of fish immunity and may inform the development of strategies for disease management in aquaculture settings.

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