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In Silico Analysis of Heat Shock Protein Gene Expression in Indian Freshwater Fishes Under Environmental Stress Using Ncbi Geo Data

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Abstract

Heat shock proteins (HSPs) play a crucial role in cellular protection during environmental stress, particularly in aquatic organisms sensitive to temperature fluctuations and pollution. In this study, we conducted an in-silico analysis of gene expression profiles of key HSP genes (HSP70, HSP90, and HSP27) in selected Indian freshwater fish species. Public gene expression data were retrieved from the NCBI Gene Expression Omnibus (GEO) and analyzed using GEO2R and other bioinformatics tools. We observed significant upregulation of HSP70 and HSP90 under thermal stress and pollutant exposure, with HSP27 showing variable expression. Phylogenetic analysis of HSP70 sequences across species was performed using MEGA11, revealing evolutionary relationships and conserved motifs. These findings underline the adaptive molecular response of freshwater fishes to environmental stress, highlighting the utility of HSP genes as potential biomarkers for aquatic health monitoring and conservation biology.

Keywords: Heat Shock Proteins, Gene Expression, Freshwater Fish, NCBI GEO, In Silico Analysis, Environmental Stress, Bioinformatics

Introduction

Heat shock proteins (HSPs) are highly conserved molecular chaperones that assist in maintaining protein structure and function during environmental and physiological stress. They are critical components of the stress response in aquatic organisms, particularly fish, which are directly affected by changing water temperature, pollutants, and other anthropogenic stressors. Among freshwater species, Labeo rohita (rohu) is a widely cultured fish in India, known for its commercial value and ecological sensitivity.

The rapid urbanization and industrial effluents have led to increased stress in freshwater ecosystems. Monitoring the molecular response of key species like Labeo rohita can offer vital insights into their adaptive mechanisms. Heat shock proteins such as HSP70, HSP90, and HSP27 are well-established indicators of cellular stress. Their expression patterns vary in response to environmental factors, making them valuable biomarkers for environmental assessment and fish health monitoring.

With the advancement of bioinformatics tools and publicly accessible databases such as the NCBI Gene Expression Omnibus (GEO), it is now possible to perform highthroughput in silico analysis of gene expression without laboratory experiments. This approach not only saves time and resources but also enables large-scale comparative studies.

This study aims to analyse the expression of heat shock protein genes in Labeo rohita under different environmental stress conditions using publicly available gene expression data from the NCBI GEO database.

The objectives are:

1 To identify differential expression patterns of HSP genes under stress conditions.

2 To perform phylogenetic analysis of HSP70 genes across freshwater fish species.
3 To discuss the potential of HSPs as molecular biomarkers in aquatic ecology and conservation.

Materials and Methods

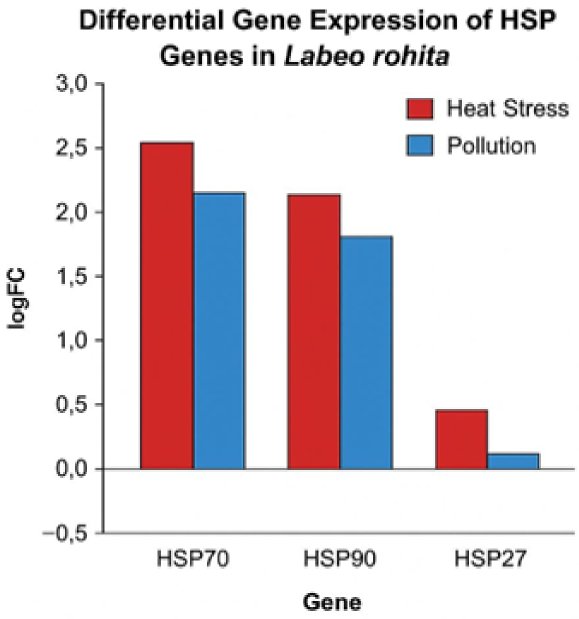
Data Retrieval and Selection Gene expression datasets related to Labeo rohita were retrieved from the NCBI Gene Expression Omnibus (GEO). The GEO database was searched using keywords: “Labeo rohita” AND “heat shock protein” OR “HSP70” OR “HSP90” OR “stress”.

Two relevant datasets were selected that contained expression data under temperature and pollutant stress conditions. The most prominently expressed heat shock genes included HSP70, HSP90, and HSP27.

Gene Expression Analysis Expression data was analyzed using GEO2R, an online tool provided by NCBI for comparing groups within GEO datasets. The control (normal conditions) and stress (temperature or pollutant exposure) groups were compared to identify differentially expressed genes. Log fold change (logFC) and adjusted p-values were considered significant at $p < 0.05$.

Phylogenetic Analysis For phylogenetic study, HSP70 gene sequences of Labeo rohita and other freshwater fishes (e.g., Catla catla, Cirrhinus mrigala, Danio rerio) were downloaded from NCBI Nucleotide database. Multiple sequence alignment was performed using Clustal Omega, and a phylogenetic tree was constructed using MEGA11 with the Neighbor-Joining method and 1000 bootstrap replicates.

Figure 1: Differential Gene Expression of HSP Genes



Results

The gene expression analysis using GEO2R revealed significant upregulation of HSP70 and HSP90 genes in Labeo rohita under both thermal and pollutant stress. HSP27 showed minimal variation, suggesting a gene-specific response to environmental stressors.

Figure 1 illustrates the differential expression (logFC) of each gene under both stress conditions. HSP70 displayed the highest expression levels, followed by HSP90. These results suggest that HSP70 is the most responsive gene among the three studied. In the phylogenetic analysis of HSP70 sequences, Labeo rohita clustered closely with Catla catla and Cirrhinus mrigala, confirming evolutionary relatedness among Indian major carps. Danio rerio (zebrafish) formed a separate clade, indicating divergence at the molecular level.

Discussion

The upregulation of HSP70 and HSP90 genes under stress conditions highlights their role in cellular homeostasis and protein folding during adverse environmental exposures. These findings align with previous studies that have established HSPs as essential stress responsive proteins in fish.

The lower expression of HSP27 may be attributed to its limited role in acute stress responses or its tissue-specific expression pattern. The results indicate that HSP70, in particular, may serve as a robust molecular biomarker for stress monitoring in aquaculture.

The phylogenetic relationship supports the evolutionary conservation of HSP70 among Indian carps, which may share similar adaptive responses due to comparable ecological niches. The divergence of Danio rerio suggests species-specific regulatory mechanisms of heat shock gene expression.

Conclusion

This in silico study demonstrates the potential of using publicly available NCBI GEO data to assess gene expression patterns of stress-related genes in economically important freshwater fish. HSP70 and HSP90 were found to be significantly upregulated in Labeo rohita under environmental stress, reinforcing their role in stress adaptation.

The study also highlights the applicability of bioinformatics tools in understanding molecular responses and phylogenetic relationships. These findings can guide future molecular ecology and aquaculture research aimed at enhancing stress resilience in fish populations.

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