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RESEARCH ARTICLE

Hsp-70 Gene Expression Analyses in the Different Ages of Rainbow Trout

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[1] *This work was the entire of master thesis entitled the expression analyses of Hsp-70 gene in the different ages rainbow trouts*

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Summary

The purpose of this study was to determinate the association between heat shock protein 70 (Hsp-70) expression and the age in rainbow trout. Total RNA was extracted with TRIzol Reagent (Life Technologies) and the first strand cDNA was synthesized using Super Script III Reverse Transcriptase. Gene amplification was done by PCR method and quantitative determination of gene expression was using beta actin gene as control. Consequently, we demonstrated that Hsp70 expression significantly increased the age.

Keywords: *Rainbow trouts (Oncorhynchus mykiss), Age, Gene expression, Heat shock protein, Hsp70*

Farklı Yaşlardaki Gökkuşağı Alabalıklarında *(Oncorhynchus mykiss)* Hsp-70 Geninin Ekspresyon Analizi

Özet

Bu çalışmanın amacı gökkuşağı alabalıklarında *(Oncorhynchus mykiss)* Hsp-70 geni ekspresyonu ile yaş arasındaki ilişkiyi belirlemektir. TRIzol Reagent (Life Technologies) ile toplam RNA,Super Script III Reverse Transcriptase ile de cDNA kütüphanesi hazırlandı. Gen amplifikasyonu PCR kullanılarak, ekspresyonun miktar belirlenmesi ise beta aktin kullanılarak yapıldı. Sonuçta Hsp-70 ekspresyonunun yaşla birlikte önemli oranda arttığı gözlemlendi.

Anahtar sözcükler: *Gökkuşağı alabalığı (Oncorhynchus mykiss), Yaş, Gen ekspresyonu, Isı şok protein, Hsp70*

INTRODUCTION

Heat shock proteins (Hsps) are a group of intracellular proteins that have an unusually high degree of identity at the amino acid level, among diverse organisms. As this family of proteins is induced by stressors other than heat, they are also commonly referred to as `stress proteins' in the literature. The term stress proteins also may refer to several other groups of proteins that respond to stressors [1].

The naming of Hsps are generally based on their molecular mass (kilodaltons, kDa). Heat shock proteins are also grouped according to function (eg chaperonin), DNA sequence, and antibody cross-reactivity. The commonly used categories are: 100 kDa; 90 kDa; 70 kDa; 60 kDa; and the 16±30 kDa group, and are usually referred to as Hsp-100, Hsp-90, Hsp-70, Hsp-60, and the low molecular weight (LMW) class of proteins, respectively [2].

In the last three decades, there has been an exponential increase in the interest and research activity concerning the description, classification and functional significance of these proteins. Heat shock proteins are constitutively expressed in cells to maintain a number of critical cellular processes relating to protein folding, fidelity and translocation. These proteins also are induced

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in cells in response to a variety of stressors and enhance survival by protecting vital cellular functions[1].

Research in this area continues to grow at an ever-increasing rate and applications to problems and opportunities in human health and environmental monitoring are developing rapidly. Heat shock protein studies in fish are still in the early stages compared with those in bacteria, yeast and mammals. Studies in fish are still in the descriptive stages of documenting novel proteins that are produced in various tissues in response to a variety of biological and abiotic stressors. For example, Hsp-70, which are expressed in response to trace metal exposure, organic pollutants changes in temperature, hypoxia, exposure to ultraviolet radiation, exercise, hyperthermia, oxidative stress, metabolic challenge, ethanol, free radicals, amino acid analogs, heavy metals and aging [3,4].

A characteristic feature of aging is a progressive impairment in the ability to maintain homeostasis in the face of environmental challenges. Age-related decline in Hsp expression may contribute to this dysfunctional homeostasis. Specifically, Hsp mRNA and protein production in response to heat shock decreases with age in a variety of species from flies to rats [5]. However, the age-related changes in Hsp response in rainbow trout, a heavily utilized research animal, have not been reported to date. Fish have long been used as an experimental model for aging [6-8]. The adaptability of the rainbow trout [9] to mutagenesis studies [10] makes it an attractive vertebrate model to study the aging process [1].

In this paper, we have evaluated effect of age on expression of Hsp-70 in muscle of rainbow trout in different age.

There are many studies involving Hsp-70 expression but none only aging [11]. Nor is there any aging study relation to Hsp-70 expression in fish. In this study, there was a relationship Hsp-70 expression and age. Thus, in this study it was seen that if fish have aging some genes can be expressed in their body such as Hsp-70. Therefore this study can become a reference for future research on relation between Hsp expression and age or plan of rearing density. And it provides information on age-related cellular and molecular changes that occur in fish species.

MATERIAL and METHODS

Fish Material

The study used different ages (8, 18 and 36 months) rainbow trout *(Oncorhynchus mykiss),* obtained from Ataturk University, Agricultural Faculty, Fishery Department Research and Extension Center Faculty of Agriculture. They were stabulated, with inconsistent mortality, at biomass density (250 kg/m^3) in a 10*2*1.3 m^3 pond connected to a water recycling system served with about 24 water refilling per day. Water parameters were strictly controlled: temperature 10±1°C, total hardness 102 mg as $CaCO_3$, free O_2 8±0.5 ppm, pH 7.8. At the average size of 8 months 150 g, 18 months 500 g and 36 months 1500 g. Food was automatically distributed daily ensured as a 1.5% body mass ratio, with extruded pellet for marine fish (Bleacksea Food). Temperature, O_2 and pH were continuously monitored, while other parameters were analyzed at a weekly rate. After 15 days, three fish for each group was randomly sampled, immediately stunned and slaughter. Skeletal muscle were removed from dorsal region, frozen in liquid N_2 and stored at 80°C until molecular biology analyses.

RNA Preperation and cDNAs Synthesis

Total RNA was isolated using TRIzol® Reagent according to the manufacturer's recommendation (Invitrogen), and the RNA pellets were resuspended in TE buffer (Tris-EDTA, pH 8.0). To eliminate possible genomic DNA contamination, the RNA samples were treated with a DNase I (Sigma) by the manufacturer's instructions. The total RNA was quantified by measuring the absorbance at 260 nm using a UV-VIS Spektro-fotometre (Aquamate), and the purity was assessed by determining the ratio of the absorbance at 260 and 280 nm. All samples had 260/280 nm ratios <1.6. Additionally, the integrity of the RNA preparations was verified by visualization of the 18S and 28S ribosomal bands stained with ethidium bromide after electro-phoresis on 1.0% agarose gels. Total RNA was reverse transcribed using a commercially available cDNA synthesis kit SuperScript™ III Reverse Transcriptase (Invitrogen). cDNA was stored at -20°C.

Quantitative RT-PCR

For PCR reaction ~100 ng template, 1X PCR buffer (1.5 mM $MgCl_2$), 200 µL of each dNTP, 0.2 µL gene-specific hsp-forward (5´- TGCACCTAGGTTTTCATAGAAT -3´) and hsp-reverse (5´- ATGGAGGTGTAGAAGTCGATGC-3´) primers, and 2.5 units of Taq DNA polymerase were mixed for a total reaction volume of 15 µL. Thermal cycling conditions were as follows: initial activation at 80°C for 1 min, at 94°C for 3 min, 40-PCR cycles at 94°C for 45s, 58°C for 45s, 72°C for 45s, and a final extension at 72°C for 5 min. For actin gene, Primers actinF (5´TGGGGCAGTATGGCTTGTATG3´) and actinR (5´ CTCTGGCACCCT AATCACCTCT-3´) were used as the internal control [11]. PCR cycles for actin gene was in initial

activation at 80°C for 1 min, at 94°C for 3 min, 35-PCR cycles at 94°C for 20s, 54°C for 20s, 72°C for 20s, and a final extension at 72°C for 5 min. The PCR products were loaded into 1% agarose gel and run in TAE 1X buffer at 100 mV for 30 min. A distinct band estimated at ~954bp nucleotides (Hsp) and ~165bp (actin) nucleotides were generated and amplified products quantified by using ImageJ 1.37c (http://rsb.info.nih.gov/ij/).

Statistical Analysis

The Student's t-test and standard deviation were used for data analysis. Significance of differences was defined as $P<0.01$ [12].

RESULTS

In the present study, we have obtained for Hsp-70 (Gen Bank accession number AB062281), and we have completed a cDNA of 3746 bp by SuperScript™ III Reverse Transcriptase (Invitrogen). We amplified a section of 954 bp of Hsp-70 gene and comprehensively compared their mRNA amplified products quantified by using ImageJ 1.37c. β-actin was amplified as 165 bp and used as a control [13], *Fig. 1*.

Quantitative mRNA levels of Hsp-70 were plotted by using gel density in 40th PCR cycle. β-actin was used as control *(Fig. 2)*. As far as quantitative mRNA is concerned, the results show that age fairly effect on fish. If it is compared with all age, quantitative mRNA levels more than ten times as high in 36 months as other age.

DISCUSSION

There are many experiments about Hsp on organisms. The expression of Hsp-70 has been studied in relation to various stressors such as heat shock [14-19], aquatic toxicants [20-25] and psychological stress [26,27]. In all of this studies, Hsp expression has been increased dramatically similar our results. However, the expression of various types of Hsp protein and mRNA levels in response to heat stress has been shown to decrease with age [18,28-33]. Pahlavani [34], showed that the induction of Hsp-70 expression by heat shock decreases 40-50% with age. There are only a few reports on the effects of aging on

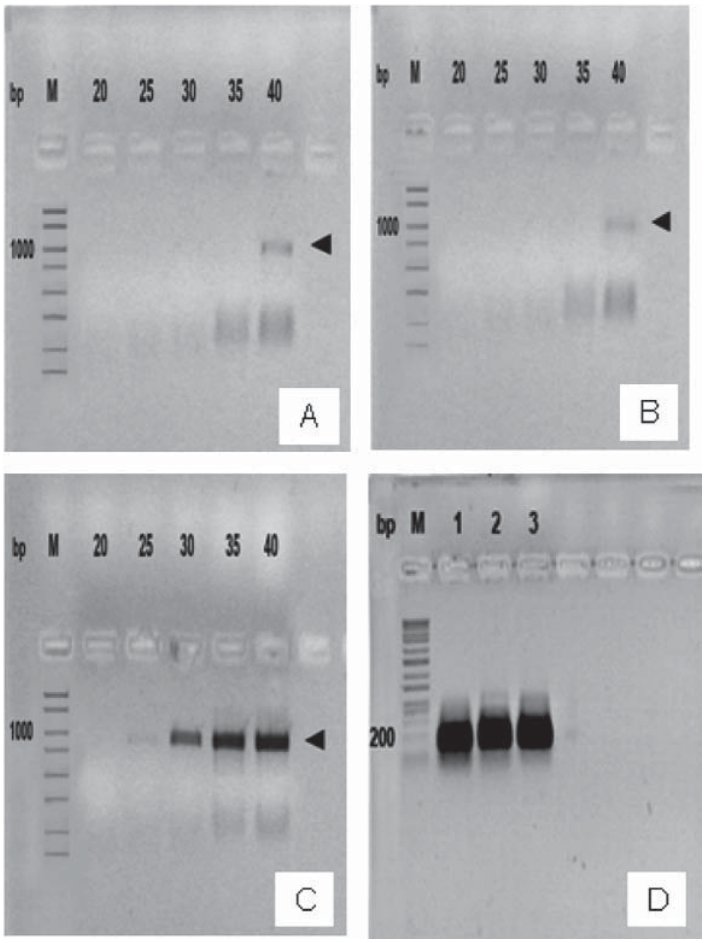

Fig 1. HSP-70 gene agarose gel patterns, 8 months **(A),** 18 months **(B),** 36 months **(C),** 20-40: PCR cycling **(A,B,C);** β-actin control: **Line 1:** 8 months, **Line 2:** 18 months, **Line 3:** 36 months **(D)**

Şekil 1. HSP-70 geninin agaroz jel görüntüleri; 8 aylık **(A),** 18 aylık **(B),** 36 aylık **(C),** 20-40 cycle **(A,B,C);** beta aktin control: **1. Sıra:** 8 aylık, **2. Sıra:** 18 aylık, **3. Sıra** : 36 aylık **(D)**

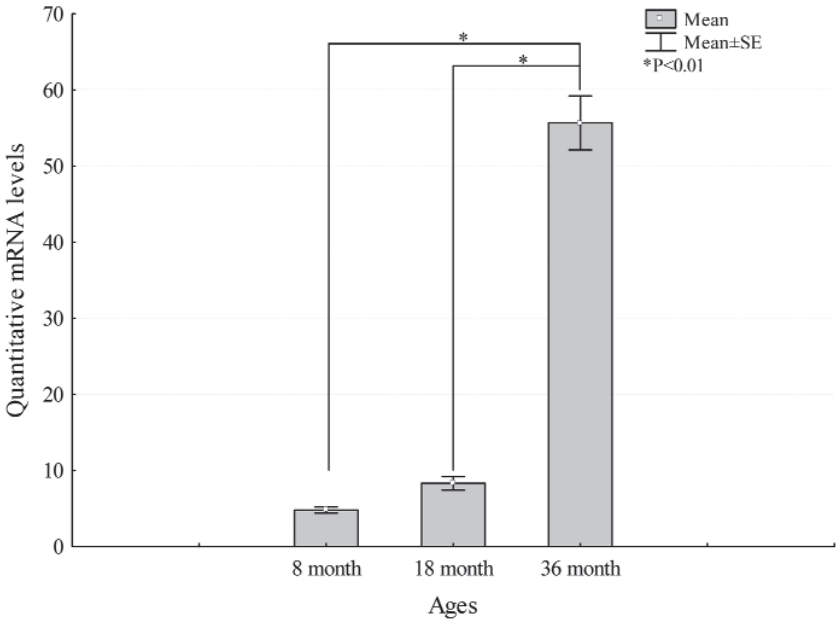

Fig 2. Relatives expression of Hsp-70 in sketal muscle of different ages groups. Asterisks denotes significant difference from age groups (P<0.01). n=7

Şekil 2. Hsp-70 geninin farklı yaş gruplarında ekspresyon ilişkisi. Şekilde yaş grupları arasında önemli farklılıklar belirlenmiştir (P<0.01). Örnek sayısı=7

fish [6-8]. These studies provide only limited information on age-related cellular and molecular changes that occur in fish species.

There are many studies involving Hsp expression. But, there isn't any experiment related with heat shock protein and age. Thus, the purpose of the present study was to isolate Hsp genes from rainbow trout and to analyze their expression profiles at the mRNA levels. In this regard, we quantitatively compared the mRNA levels between different ages under rearing the same condition using PCR analysis. The present work demonstrated the expression of Hsp-70 gene was increased with fish age.

In this study the effect of age on Hsp-70 gene expression in different age fish was investigated. Consequently, Hsp-70 gene expression increase with fish age.

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