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Use of heat shock protein mRNA expressions as biomarkers in wild crucian carp for monitoring water quality

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ABSTRACT

In organisms, the production of heat shock proteins (HSPs) can be induced after exposure to various stressors. In the present study, partial cDNA sequences encoding of HSP30, HSP60, HSP70, and HSP90 genes were isolated and sequenced in the crucian carp (*Carassius carassius*). Next, quantitative real-time reverse transcription PCR (qRT-PCR) assays normalized to beta-actin were developed to quantify HSP mRNA expression. The same methods were used to detect HSP mRNA expression in the wild crucian carp that were collected from the Hun River. In fish located downstream of the river, we found that the levels of HSP70 and HSP30 in the liver and kidney were higher than fish located upstream, and these differences coincided with changes of the water quality. These results suggest that kidney HSP30 and liver HSP70 expression can serve as sensitive biomarkers for the presence of field environmental stressors in wild crucian carp.

1. Introduction

Heat shock proteins (HSPs) are stress proteins that are involved in the protection and repair of cells following cellular damage. They are categorized into several major families according to their molecular weight. Our knowledge of their function at the molecular and cellular level is increasing (Khalil et al., 2011; Sung and MacRae, 2011; Tower, 2011; Carra et al., 2012; Ghasemi et al., 2012; Lee et al., 2012), and more studies are focusing on the HSPs as biomarkers for indicating general environmental stressors (Robert et al., 2010). When organisms are exposed to contaminants, the properties and expression patterns of their HSPs are altered, potentially enabling HSPs to be used to gauge the effects of pollutant exposure during biomonitoring studies (Golli-Bennour and Bacha, 2011; Kafel et al., 2011; Wang et al., 2011a,b; Chankova et al., 2012; Lauritano et al., 2012) and environmental risk assessment (Lewis et al., 1999; Bierkens, 2000).

Laboratory studies conducted under controlled conditions have shown that HSP responses can be induced in fish after exposing them to various individual or combined factors, such as pesticides and heavy metals (Varo et al., 2002; Airaksinen et al., 2003), abnormal temperatures (42.5 °C) (Wagner et al., 1999), salinity (Deane et al., 2002), anoxia (Airaksinen et al., 1998), oxidative stress (Broome et al., 2006), and/or bacterial infections (Mayr et al., 2000), all of which can affect water quality and the aquatic environment. Moreover, some field investigations have also examined the ability of HSPs to be used as biomarkers of pollution in lake (Wang et al., 2007), stream (Triebskorn et al., 2002), and coastal area (Schulte, 2007), and these studies have reported that HSP levels in the fish collected from polluted areas were higher than those in the unexposed control fish (Wang et al., 2007). Although no

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Table 1 – Basic parameters of wild crucian carp from Hun River.

Sites	Length (cm)	Weight (g)	GSI (%)	CF
S1	12.50 + 1.78	34.07 + 13.48	9.63 + 3.09	1.67 + 0.11
S2	15.60 + 0.81	51.27 + 13.44	12.98 + 3.30	1.92 + 0.08
S3	13.78 + 0.85	48.85 + 9.47	7.69 + 4.48	1.85 + 0.16
S4	17.10 + 1.29	42.60 + 20.18	11.42 + 2.92	1.62 + 0.08
S5	14.05 + 1.08	38.12 + 7.91	10.45 + 1.34	1.38 + 0.16
S6	14.15 + 1.90	57.76 + 24.79	12.73 + 2.54	1.94 + 0.24
S7	15.42 + 0.93	63.25 + 17.34	13.52 + 7.55	1.69 + 0.28

Notes: Gonadosomatic index (GSI) (%) = gland weight (g) × 100/body weight(g); condition factor (CF) = body weight(g) × 100/[total length(mm)^3].

individual contaminant was shown to correlate with HSP levels in these investigations, changes in HSP level could reflect the effects of low-level contamination by heavy metals or polycyclic aromatic hydrocarbons (Webb and Gagnon, 2009). Therefore, these proteins or mRNA expression may provide early warning information on the biological effects of pollution but not a specific pollutant.

The Hun River is one of main tributaries of the Liao River with a drainage area of 11,481 km^2. In the river basin, which is characterized by a fast-growing economy development, a large volume of treated and untreated wastewater, containing pollutants, is discharged into the Hun River from surrounding cities such as Shenyang, Fushun, and Anshan. Hence, water quality of the Hun River has seriously deteriorated in recent years, which has had a negative impact on the ecological integrity of the Hun River (Wang et al., 2011a,b). Therefore, it is crucial to understand the physiological responses of native organisms inhabiting the river for early warning its potential ecological risk.

In the present study, we isolated and sequenced several HSPs gene, namely HSP30, HSP60, HSP70, and HSP90 in the crucian carp (*Carassius carassius*). A quantitative reverse-transcription polymerase chain reaction (qRT-PCR) assay was then conducted to determine their expressions in wild fish for evaluating the water quality.

2. Materials and methods

2.1. Sampling

In this investigation, site 1 (S1) was located upstream of the Dahuofang reservoir and was considered to be the reference site. Sites 2 and 3 (S2 and S3) were located in the environs of Fushun city; sites 4 and 5 (S4 and S5) were in the surrounding area of Shenyang city; and two further sites (S6 and S7) were downstream away from the city, as shown in Fig. 1.

In July 2010, 12–22 wild crucian carp specimens were collected from each sampling sites in the Hun River using fishing nets, and then their body weights and lengths were measured (Table 1). After that, parts of gill, liver, and kidney tissues were frozen in liquid nitrogen as quickly as possible until further analysis. At the same time, water quality parameters namely pH, temperature and dissolved oxygen (DO) were measured *in situ* using YSI (DR820, HACH, USA), and TP (total phosphorus), NH_3-N, biochemical oxygen demand (BOD_5) and chemical

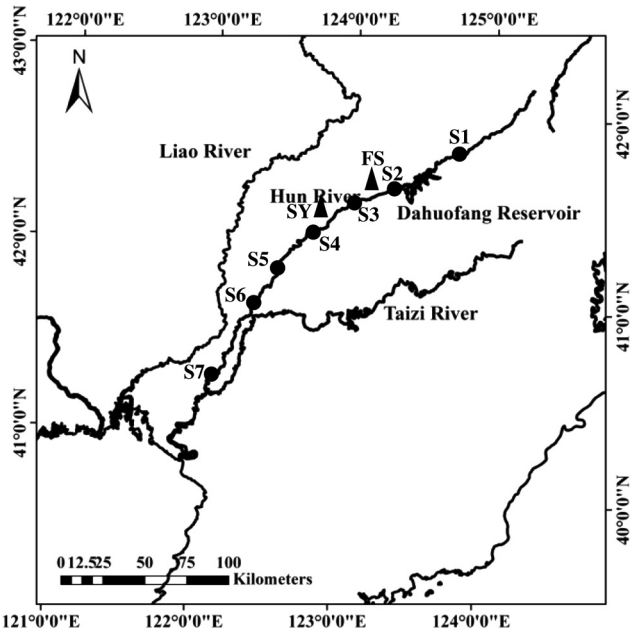

Fig. 1 – Sampling sites (S1–S7) in Hun River. SY: Shenyang city; FS: Fushun city.

oxygen demand (COD_{Cr}) were measured using the standard methods (Table 2).

2.2. Total RNA isolation and first strand cDNA synthesis

Total RNA was RNA extraction for 20–30 mg of tissues was done with Trizol® Reagent (Invitrogen Ltd., NY, USA). Next, RNA samples were incubated at 37 °C with RNase-free DNase I (TakaRa Ltd., Dalian, China), and then re-suspended in 50–100 μl diethylpyrocarbonate-treated water. RNA concentrations and purity were determined spectrophotometrically taking optical density (OD) at 260 and 280 nm. Then, about 1 μg total RNA was reverse transcribed for first strand cDNA synthesis using SuperScript III kit (Invitrogen), according to the manufacturer's instructions. First strand cDNA samples were then diluted 10 times with sterile double-distilled water and stored at −20 °C for PCR analysis.

2.3. Amplification and sequencing of HSPs

According to the principle of degenerate primer, degenerate PCR primers were designed utilizing BioEdit 7.0 programs, and then were used to amplify the conserved fragment of HSPs cDNA (Table 3). These primers were designed around highly conserved sequences located near the N and C terminus of HSPs according to the alignments of available HSPs mRNA sequences of fishes and other organisms available in NCBI database (http://www.ncbi.nlm.nih.gov/). The amplification conditions, performed in 50 μL reaction volumes, were 1 min at 95 °C for initial denaturation, followed by 40 cycles at 95 °C for 30 s, 55–56 °C for 30 s, and 72 °C for 1 min. The products were separated on a 1.2% agarose gel, and the amplicon was purified, cloned, and sequenced. The resultant nucleotide