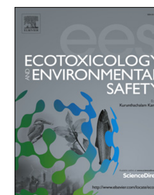
Ecotoxicology and Environmental Safety 126 (2016) 30–37

Contents lists available at ScienceDirect

Ecotoxicology and Environmental Safety

journal homepage: www.elsevier.com/locate/ecoenv



Characterization of antioxidant system parameters in four freshwater fish species

Gülüzar Atli [a,*], Esin G. Canli [b], Ali Eroglu [b], Mustafa Canli [b]

[a] Cukurova University Vocational School of Imamoglu, Adana, Turkey
[b] Cukurova University Faculty of Science, Department of Biology, Adana, Turkey

ARTICLE INFO

Article history:
Received 13 August 2015
Received in revised form
9 December 2015
Accepted 10 December 2015
Available online 18 December 2015

Keywords:
Characterization
Antioxidant enzymes
Glutathione
Freshwater fish

ABSTRACT

The potential use of antioxidant system parameters has gained considerable interest due to their pivotal role of detoxification mechanisms in environmental studies and culture fish point of view. Fish with different ecological needs may have different antioxidant capacity and response to environmental contaminants. Thus, the optimal working conditions and specific enzyme activities (V_{max} and K_m) of antioxidant system parameters (Superoxide dismutase, SOD; Catalase, CAT; Glutathione peroxidase, GPX; Glutathione reductase, GR and Glutathione S-transferase, GST) and glutathione (GSH) were determined in four commonly cultured freshwater fish species (tilapia; *Oreochromis niloticus*, carp; *Cyprinus carpio*, trout; *Onchorhynchus mykiss* and catfish; *Clarias garipienus*). Data showed that optimal concentrations of different buffers, pH and specific chemicals for each enzyme and GSH were similar in most cases for all fish species, except a few differences. The highest V_{max} and K_m values were found in carp for GPX and GST, though these values were the highest in tilapia, catfish and trout for CAT, SOD and GR, respectively. As a conclusion, optimization assays of these parameters in different bioindicator organisms based on their physiological and ecological differences may be useful for the aquatic ecosystem biomonitoring studies and also present fundamental data for utilization in aquaculture.

© 2015 Elsevier Inc. All rights reserved.

1. Introduction

Reactive oxygen species (ROS) such as hydrogen peroxide, superoxide radical, hydroxyl radical leading to oxidative stress in fish (Radi and Matkovics, 1988; Roche and Boge, 1993; Dautremepuits et al., 2002). The oxidative stress has gained considerable interest in the field of ecotoxicology. Antioxidant enzymes are crucial in the effort to counteract oxidative stress caused by toxicants once the supply of other antioxidant compounds are depleted (Radi and Matkovics, 1988; Martinez-Alvarez et al., 2005). Antioxidant enzymes, such as GPX, GST, GR and SOD are important in coping with oxidative stress caused by metabolisms itself and environmental factors (Pinto et al., 2003; Tripathi et al., 2006). SOD catalyzes the reduction of superoxide radical into hydrogen peroxide which is eliminated by CAT into oxygen and water. GPX also participates in the reduction of hydrogen peroxide. On the other hand, GST catalyzes the conjugation of pollutants to eliminate them from the cellular system and GR reduces the oxidized glutathione to GSH which acts as an electron donor. The antioxidant parameters are often employed in ecotoxicological studies, as they can give sensitive data about the stress that fishes face. Studies have shown that metals present in water are able to alter the levels of the antioxidant system enzymes in the liver of freshwater fishes (Atli and Canli, 2010; Kanak et al., 2014). The liver is the site of multiple oxidative reactions and maximal free radical generation and it is the organ to eliminate xenobiotic. It was frequently referred to since it is known to best represent antioxidant defense status in vertebrates with its high metabolism and oxygen consumption (Davies, 1991; Wilhelm-Filho et al., 1993; Atli et al., 2006; Atli and Canli, 2010). A strong recommendation for chemical data integration with biomarkers was made to characterize the contaminant effect on aquatic organisms exposed to xenobiotics (Barata et al., 2005). It was shown that antioxidant enzymes are significantly influenced by several toxicants in fish species (Wong and Wong, 2000; Sanchez et al., 2005; Atli and Canli, 2010; Eroglu et al., 2015). Therefore, characterization of these parameters in liver tissue is important and also essential to obtain accurate data, especially in environmental monitoring studies (Hidalgo et al., 2002; Avci et al., 2005).

Nile tilapia (*O. niloticus*) is regarded as one of the most saline tolerant teleost up to 10 ppt (Kamal and Mair, 2005; Baysoy et al., 2013) and the optimum water temperature range is approximately 24–28 °C. Their feeding behavior is based mostly on aquatic invertebrates, detritus, and plankton (Popma and Masser, 1999;

* Corresponding author.
E-mail address: gatli@cu.edu.tr (G. Atli).

http://dx.doi.org/10.1016/j.ecoenv.2015.12.012
0147-6513/© 2015 Elsevier Inc. All rights reserved.

Kamal and Mair, 2005). The common carp (*C. carpio*) is a stenohaline freshwater fish (0–6 ppt) (Mangat and Hundal, 2014) for which the optimal temperature for growth is between 22–26 °C and it mainly feeds on detritus, plants, and benthic organisms (Maitland and Campbell, 1992; Metz et al., 2003). On the other hand, the natural habitat water temperature for rainbow trout, *O. mykiss*, is around 12 °C, which is relatively low when compared to other species. It generally feeds on aquatic mollusks, crustaceans, and other small fishes (Gall and Crandell, 1992). African catfish (*C. gariepinus*) which has the optimal temperature around 25–30 °C for growth (Britz and Hecht, 1987) and salinities up to 10 ppt (Safriel and Bruton, 1984) is of great commercial importance because it is the most common fresh water fish widely consumed. It is regarded as one of the most suitable species for aquaculture due to its fast growth (Bruton, 1978; Spataru et al., 1987), flexibility in its trophic behavior (Bourn, 1973), its hardiness to withstand extreme environmental conditions (Groenewald, 1964) and its feeding on a wide variety of foods such as detritus, filamentous algae, zooplankton, macrophytes, insects, nematodes, mollusks, crustaceans and fish (Corbet, 1961; Bourn, 1973; Bruton, 1978).

In this study, the optimal working conditions of antioxidant system parameters were determined in the liver tissues of four freshwater fish with different ecological needs (tilapia, catfish, carp and trout). The present data were compared with the literature concerning other fish species and evaluated from the aquaculture point of view.

2. Materials and method

Adult freshwater fish (*O. mykiss*, *O. niloticus*, *C. carpio* and *C. gariepinus*) were obtained from fish culturing pools in the Cukurova region (Adana, Turkey). The physicochemical properties of culture waters were measured at the time of fish sampling. The pH, temperature and oxygen levels were in the range of 7.27–7.79, 22.1–23.8 °C and 6.21–6.30 mg O_2/mL, respectively. However the water temperature was 11 °C for *O. mykiss*. Total hardness (with EDTA titration method) and alkalinity (acidimetry method) were measured in the range of 336–366 mg $CaCO_3$/L and 315–340 mg $CaCO_3$/L, respectively whereas total hardness was found as 298 mg $CaCO_3$/L for *O. mykiss*. In addition, conductivity of the waters was in the range of 610–631 μS/cm except the culture water of the *O. mykiss* which was 554 μS/cm. A total of 8 fish for each species were killed after taking them from the culture pools according to the decision of an Ethic Committee of Cukurova University. Liver tissues were dissected by using clean equipment and stored at −80 °C until the analysis. The tissues were homogenized (1:10, w/v) in homogenization buffer containing 100 mM potassium phosphate buffer (pH 7.4), 100 mM KCl and 1 mM EDTA at 9500 rpm for 1.5 min. Homogenates were centrifuged at 10,000*g* (Hettich Universal 30 RF) for 30 min (+4 °C). Supernatants were used for the analysis. All chemicals used in this study were obtained from Sigma or Merck (Germany). Total lengths and weights of trout, tilapia, carp and catfish were measured as 30.2 ± 0.5 cm and 291.5 ± 11.0 g, 15.0 ± 0.4 cm and 150.0 ± 29.2 g, 22.2 ± 1.2 cm and 60.0 ± 11.5 g, 24.0 ± 1.4 cm and 48.3 ± 3.04 g, respectively.

2.1. Enzyme activity assay

The CAT activity was measured at 240 nm, using a specific absorption coefficient at 0.0392 cm^2 μmol^{-1} H_2O_2. One mL of the substrate solution was made up of 25 mM H_2O_2 in a 75 mM phosphate buffer at pH 7.0 and 20 μL of the supernatant. The absorbance decrease was monitored for 1 min expressed as μmol H_2O_2 decomposed/mg prot./min. The GPX activity was measured by using the method of Livingstone et al. (1992) and expressed as μmol/mg prot./min. The activity was calculated estimating the decrease of NADPH at 340 nm for 1 min. The reaction buffer contained 100 mM phosphate buffer (pH7.4), 2 mM GSH, 0.12 mM NADPH, 2 U Glutathione reductase, supernatant and 3 mM cumene hydroperoxide in a final volume of 1 mL. The GR activity was also calculated by the decrease of NADPH at 340 nm for 1 min and expressed as μmol/mg p/min. (Carlberg and Mannervik, 1975). The reaction medium contained 100 mM phosphate buffer (7.4), 0.1 mM NADPH and 0.1 mL supernatant. The reaction was initiated by adding 1 mM GSSG in a final volume of 1 mL. SOD activity was measured by the indirect method involving the inhibition of cytochrome c reduction at 550 nm for 1 min (McCord and Fridovich, 1969). The reaction buffer contained 50 mM potassium phosphate buffer (pH 7.8), 0.1 mM EDTA, 10 mM cytochrome c, 0.05 mM hypoxanthine and the supernatant. The reaction was started by adding 1.87 mU/ml Xanthine oxidase in a final volume of 1 mL. A unit of an SOD activity was defined as the amount of enzyme that causes 50% inhibition of cytochrome c reduction and was expressed as Unit/mg protein. The GST activity was evaluated by an absorbance increase at 340 nm, resulting from the conjugation of reduced glutathione (GSH) and CDNB (1-chloro-2,4-dinitrobenzene) (Habig et al., 1974). The reaction buffer contained 100 mM potassium phosphate buffer (pH 7.4), 1 mM GSH, 1 mM CDNB and the supernatant in a final volume of 1 mL. The GST activity was expressed as μmol/min/mg prot. GSH levels were measured by the absorbance change at 412 nm for 1 min and expressed as μmol GSH/mg prot./min. (Griffith, 1980). The reaction buffer contained 143 mM sodium phosphate buffer (pH 7.5), 0.3 mM NADPH, 6 mM DTNB, 50 U/mL GR and the supernatant in a final volume of 1 mL. The protein contents of the homogenates were determined by the method of Lowry et al. (1951), using bovine serum albumin as a standard. The characterization studies were made in an air-conditioned room, keeping its temperature at 25 ± 1.0 °C during the analysis. The concentration ranges of the incubation media of the studied antioxidant parameters were selected based on the previous data (Nagai et al., 2002; Sen and Kirikbakan, 2004; Barata et al., 2005).

3. Results

SOD activity was measured maximally at 75 mM phosphate buffer concentration in all fish species, except the carp (100 mM) (Fig. 1.). The optimal pH value was 7.0 for trout, whereas it was 7.5 for the other species. The maximal SOD activity was measured at 1.5 mM hypoxanthine for carp, catfish and trout, while it was 1.875 mM for tilapia. Optimal cytochrome c concentration was observed at 0.1 mM for trout and carp, though it was 0.2 mM for tilapia and catfish. SOD activity in catfish and trout was measured maximally at 56.6 mU/mL, whereas it was at 113.2 mU/mL for tilapia and carp. The highest specific enzyme activity (V_{max}) and K_m values were measured as 34.6 U/mg prot. and 0.00696 mM in catfish (Table 1).

Optimal phosphate buffer concentration and pH values for CAT activity were observed at 75 mM and pH 7.0, respectively in all species with an exception of catfish (pH: 7.5) (Fig. 2). In addition, maximal CAT activity for all species was measured at 25 mM H_2O_2 concentration, though it was 35 mM for catfish. *O. niloticus* showed the highest V_{max} (27.2 μmol H_2O_2/mg prot/h) and K_m values (0.065 mM) (Table 1).

Maximal GPX activity was measured at pH 7.0 and 100 mM phosphate buffer concentration with an exception of tilapia (125 mM) (Fig. 3). Optimal NADPH and GSH concentrations were found as 4.8 and 60 mM, respectively for all species. The highest enzyme activity in catfish and carp was determined at 30 mM GR concentration, whereas it was 40 mM GR concentration for tilapia

Download English Version:

https://daneshyari.com/en/article/4419205

Download Persian Version:

https://daneshyari.com/article/4419205

Daneshyari.com