

# Antioxidant enzyme characterization in the liver of Mediterranean barbel (*Barbus meridionalis*) from Osor river (Catalonia)

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## Abstract

Antioxidant system parameters have still gained considerable importance due to their pivotal role in detoxification mechanisms. Optimization assays were carried out for antioxidant enzymes (Superoxide dismutase (SOD); Catalase (CAT); Glutathione peroxidase (GPX); Glutathione reductase (GR) and Glutathione S-transferase (GST) in the liver of Mediterranean barbel (*Barbus meridionalis*). The characterization of the antioxidant enzymes was carried out for several incubation media parameters. For the pH optimization, the range of 6.0-8.0 was assayed and the maximal enzyme activities were found at pH 7.0 for SOD and GPX and pH 7.5 for the CAT, GST and GR. Phosphate buffer concentrations in the range of 50-150mM were examined and generally, all enzymes showed their highest activities at 100mM phosphate buffer except SOD activity which was maximally at 150mM. Specific enzyme activity ( $V_{max}$ ) and  $K_m$  values were also determined. Optimal values of other incubation media for each antioxidant enzyme were mostly found in similar ranges when compared to several fish species in the literature. Characterization assays of these parameters in native fish based on its physiological and ecological importance may be useful for biomonitoring of aquatic ecosystems health and also present fundamental data for utilization in further studies in the area of ecotoxicology.

In this study, barbel was chosen as a suitable test-organism in biomonitoring studies owing to its wide geographical distribution and benthic way of life. Barbel has an intestinal parasite *Pomphorynchus laevis* (*Acanthocephala*). It has not been widely investigated in the study of AOS but literature data show that it is very useful for biomonitoring studies in aquatic ecosystems. Barbel represents the final host of the adult worms that live inside its intestine. Fish become infected when feeding on crustaceans that contain infective larvae in their hemocoels. The liver was chosen for the present study due to its responsibility for the regulation of overall body metabolism and thereby intense involvement in the detoxification of xenobiotics. The muscle is important because of its consumable value. The parasite was chosen because some literature data show that it is very useful as a bio indicator in heavy metal pollution

Thus, the evaluation of antioxidant responses in these tissues and parasite is highly relevant since toxic chemicals, causing temporary or permanent disturbance of homeostasis, can disrupt their functions.

The aim of this study was to investigate and compare the activities of total superoxide dismutase (Tot-SOD, EC 1.15.1.1), manganese-containing Superoxide dismutase (Mn-SOD), copper/zinc-containing superoxide dismutase (Cu/Zn-SOD) and catalase (CAT, EC 1.11.1.6), the total protein concentration, as well as protein and SOD electrophoretic profiles in the liver and muscle of barbel (*Barbus barbus*, L.) and in its intestinal parasite *Pomphorynchus laevis* from the Danube river. The metabolic activities of fish are influenced by changes in abiotic and biotic factors that depend on season. All enzyme activities were measured simultaneously in triplicate for each sample using a Shimadzu UV-160 spectrophotometer and a temperature controlled cuvette holder. The activity of Tot-SOD was assayed by the epinephrine method based on the capacity of SOD to inhibit autoxidation of adrenaline to adreno chrome. One unit of SOD activity was defined as the amount of protein causing 50% inhibition of the autoxidation of adrenaline at 26°C. The activity of Mn-SOD was obtained after the inhibition of Cu/Zn-SOD with KCN. Cu/Zn-SOD activity was calculated as a difference between TotSOD and Mn-SOD activities. Total protein concentration was determined according to the method of Lowry et al. (1951) using bovine serum albumin as a reference and expressed in mg/mL protein. Protein electrophoretic profiles were examined by the standard method of sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE). SOD electrophoretic profiles were examined using nitroblue tetrasolium (NBT). The enzyme system SOD-CAT represents the first line of defense against free radicals. SOD catalyzes the dismutation of the superoxide anion radical. As a result,  $H_2O_2$  is produced and decomposed by the CAT. It is usual to expect a simultaneous induction of SOD and CAT, but not in our case. In the present study, the SOD level was low in summer and the CAT level was high whereas the SOD level was high in spring and the CAT level was low. The low levels of CAT could be attributed to an increased production of superoxide anion radical which has been reported to inhibit CAT activity in case of excessive. It should be emphasized that barbell adults spawn once a year in mid- or late spring

therefore, these changes in enzyme activities could also implicate the intense reproductive activity that occurs in that period. during the annual reproductive cycles of fish. In addition to seasonal variations, reproductive status and pollution, other factors also have a great impact on the physiological levels of these biomarkers and could also explain some of the variations observed for AOS components and protein concentration. These factors are food availability (dissolved oxygen concentration (age light intensity and swimming activity Antioxidant enzymes are rarely investigated in parasites. These enzymes are essential for parasites to defend themselves against the ROS generated by the macrophages, neutrophils and eosinophils of the host, in addition to their normal functions in aerobic organisms. These enzymes may be particularly important for long-lived parasites. In our study, we found seasonal changes in SOD and CAT activities in the parasite *Pomphoryinchus laevis*. Both these enzymes had the same trend in activity as the enzymes in barbell tissues.

We assume that these changes in the enzyme activities in a parasite could be a consequence of seasonal changes in its host. The investigation of this enzyme system represents a major advance towards the understanding of how parasitic acanthocephalans deal with both internal and environmental oxidative stress. The analysis of protein and SOD electrophoretic profiles shows strong seasonal characteristics in the liver and muscle of the barbel *Barbus barbus* and the parasite *Pomphoryinchus laevis*. In conclusion, to our knowledge, this is the first report on SOD and CAT activities in the liver and muscle of barbel and in its intestinal parasite *Pomphoryinchus laevis* on the Serbian bank of the Danube. From the presented results it can be concluded that the seasonal pattern of AD found in barbs *Barbus* seems to be closely correlated with the seasonal variations of temperature and the reproductive cycle.

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