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Evaluation of stress related enzymes in the marine crab *Portunus pelagicus*

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Abstract

The crustacean crab metabolism is unique and inadequately in demanding the repose on the enzyme which restores the functioning of the crab. The extrinsic and intrinsic factors of these enzymes were active and collective functioning of entire metabolism. The standard assay techniques to determine the free radical and percentage of inhibition of the enzyme sample were quantified. The *Portunus pelagicus* enzymes of superoxide dismutase, catalase and glutathione S-transferase were studied and their activity is recorded for the three different location of identical crab. The Sample locations were Kanchipuram, Rameshwaram and Therespuram. The free radical activity percentage of approximately 23% to all the crab samples tested. The SOD analysis showed the activity in IC50 value in Rameshwaram sample of about 18.33 U/ml respectively.

Keywords: *Portunus pelagicus*, Catalase, SOD and IC50

Introduction

Crab culturing shows importance from ancient period due to demand in live crabs and its productivity. Most of the edible crabs from brackish water and marine environments with five genera of the portunidae family were reported (John Samuel *et al.*, 2004). Annual marine crab landings in India were increased during 1977-2005 on which *P. pelagicus* contribute about 35% and countries like Japan, Philippines, Indonesia, Bangladesh and Australia were actively involves in crab culturing (Josileen Jose, 2006 and Soundarapandian *et al.*, 2007).

To date, several studies have been focused on understanding the biology of marine crustaceans, particularly of commercially important species such as shrimp, crabs and lobsters, whose physiological abilities to face environmental stress have been demonstrated through their permanence in the marine ecosystem. Previous evidence confirms that crustaceans under conditions of osmotic stress may display response mechanisms such as accumulation of a variety of organic solutes (osmolytes) in an effort to counteract the resulting movement of water. These osmolytes may include free amino acids, polyhydric alcohols, quaternary ammonium or tertiary sulphonium compounds (Jahn *et al.*, 2006)

Besides being the largest and most dynamic reservoir of biomass on Earth, Oceans are the ultimate repository for a vast amount of discharged compounds via human activities (Kennish, 1996). In addition, marine dynamics promote continuous changes in environmental factors such as temperature, salinity and dissolved oxygen concentration, which, in addition to the presence of marine pollutants, significantly affects marine wildlife. The deterioration of the environmental conditions in the oceans may eventually lead to warming. Eutrophication, acidification, oxygen depletion (hypoxia) and the accumulation of toxic compounds as ammonia and sulfides (Fabry *et al.*, 2008 and Hoegh-Guldberg and Bruno, 2010). A long list of toxic contaminants has been found affecting the marine ecosystem. Oil, gas and agricultural wastes are among the most studied pollutants affecting the marine environment. In addition, bioactive metabolites synthesized by microalgae in marine ecosystems, as various unsaturated aldehydes, are among the most toxic compounds (Leflaive and Ten-Hage, 2009).

Oxidative stress is considered as an impotant biomarker of the health status of organisms (Halliwell and Gutteridge, 2001). It is a well known fact that fluctuations in both biotic and abiotic components of the marine environment have considerable impact on the metabolic activities of its inhabitants particularly of the invertebrates (Abele and Puntarulo, 2004; Lesser, 2006; Almeida *et al.*, 2007). Oxidative stress of an organism is a reflection of its metabolic state.

This marine organism is sensitive to several aquatic contaminants and adopts several defense mechanism against any environmental chemical, physical and mechanical stress, in an attempt to preserve the developmental program. The sea urchin embryo represents a suitable model system to investigate the adaptive response of cells exposed to stress during development and differentiation (Roccheri *et al.*, 2004; Agnello *et al.*, 2007).

Environmental stress that threaten the functional integrity of an organism result in the expression of a set genes the protein products of which serve to protect the organism from the deleterious effects of the stress (Parsell and Lindquist, 1993; Feder and Hoffman, 1999).

Catalase is considered by many scientists as an important and sensitive biomarkers of oxidative stress than Super oxide dismutase, revealing biological effects on the redox status of the marine organisms (Regoli *et al.*, 2002 a,b). A range of other environmental stresses have been shown to induce HSPs, and the term “stress proteins” has also been used to describe these proteins (Lewis *et al.*, 1999). There are many reports that evidence the expression of HSPs in response to environmental stresses (Feder and Hoffman, 1999; Qari, 2004; Lockwood *et al.*, 2010; Schoville *et al.*, 2012).

A variety of environmental contaminants and their metabolites have toxic effects associated with oxidative stress. Thus the analysis of oxidative biomarkers can help to evaluate environments contaminated by complex mixtures of xenobiotics. Hence the present study has been carried out to compare the expression of selected markers of oxidative stress of the blue swimming crab *P. pelagicus*. Investigations were made for the enzymatic (superoxide dismutase-SOD, catalase-CAT) biomarkers of oxidative stress.

Materials and Methods

The stress enzymes of the crab were assayed for its activity and its inhibition concentration based on sample amount processed for its sample preparation.

Estimation of Catalase enzyme assay

Catalase activity was estimated by the method of Sinha 1972, and reagents were prepared based on its concentration and free radicals activity were calculated.

Reagents

Standard: Hydrogen peroxide

Phosphate buffer (0.01 M, pH 7.0)
5% Potassium dichromate was prepared with dilute acetic acid (1:3 W/V in distilled water)
Hydrogen peroxide

Procedure

To 0.01 ml of the sample, 1ml of phosphate buffer, 1 ml of hydrogen peroxide was added and the timer was started. The reaction was arrested by the addition of 2 ml Dichromate-acetic acid reagent. Standard Hydrogen peroxide in the range 4 - 20 µM were taken and treated similarly, the tubes were heated in the boiling water bath for 10 min. The green colour

developed was read at 570 nm using a spectrophotometer, and percentage of free radical availability was calculated by the following formula,

$$\text{\% of free radicals available in the sample} = \frac{\text{(Absorbance of control – absorbance of sample)}}{\text{Absorbance of control}} \times 100$$

Superoxide dismutase assay

The Calbiochem® Superoxide Dismutase Assay Kit II utilizes a tetrazolium salt for detection of superoxide radicals generated by xanthine oxidase and hypoxanthine. One unit of SOD is defined as the amount of enzyme needed to exhibit 50% dismutation of the superoxide radical. The SOD assay measures all three types of SOD (Cu/Zn-, Mn-, and Fe-SOD). The assay provides a simple, reproducible, and fast tool for assaying SOD activity in plasma, serum, erythrocyte lysates, tissue homogenates, and cell lysates.

Procedure

Preparation of SOD standards

Dilute 20 µl of the SOD standard with 1.98 ml of 1X Sample Buffer to obtain the SOD stock solution. Take seven clean glass test tubes and mark them A-G. Add the amount of SOD stock and 1X Sample Buffer to each tube as described in the below Table.

Tube	SOD stock (µl)	Sample Buffer (µl)	Final SOD Activity (U/ml)
A	0	1000	0
B	20	980	0.025
C	40	960	0.05
D	80	920	0.1
E	120	880	0.15
F	160	840	0.2
G	200	800	0.25

SOD Standard Wells

Add 200 µl of the diluted radical detector and 10 µl of standard (tubes A-G) per well in the designated wells on the plate

Sample Wells

Add 200 µl of the diluted radical detector and 10 µl of sample to the wells Reaction is initiated by adding 20 µl of diluted xanthine oxidase to all the wells you are using. Carefully shake the 96 well plate for a few minutes to mix. Cover with the plate cover and incubate the plate on a shaker for 20 min at room temperature. Read the absorbance at 450 nm using a microplate reader.

Calculation

Determination of the Reaction Rate

1. Calculate the average absorbance of each standard and sample.
2. Divide standard A's absorbance by itself and divide standard A's absorbance by all the other standards and samples absorbance's to yield the linearized rate (LR) (i.e., LR for Std A = Abs Std A/Abs Std A; LR for Std B =Abs Std A/Abs Std B).
3. Plot the linearized SOD standard rate (LR) (from step 2 above) as a function of final SOD Activity (U/ml) from
4. Calculate the SOD activity of the samples using the equation obtained from the linear regression of the standard curve substituting the linearized rate (LR) for each sample. One unit is defined as the amount of enzyme needed to exhibit 50% dismutation of the superoxide radical.

Results

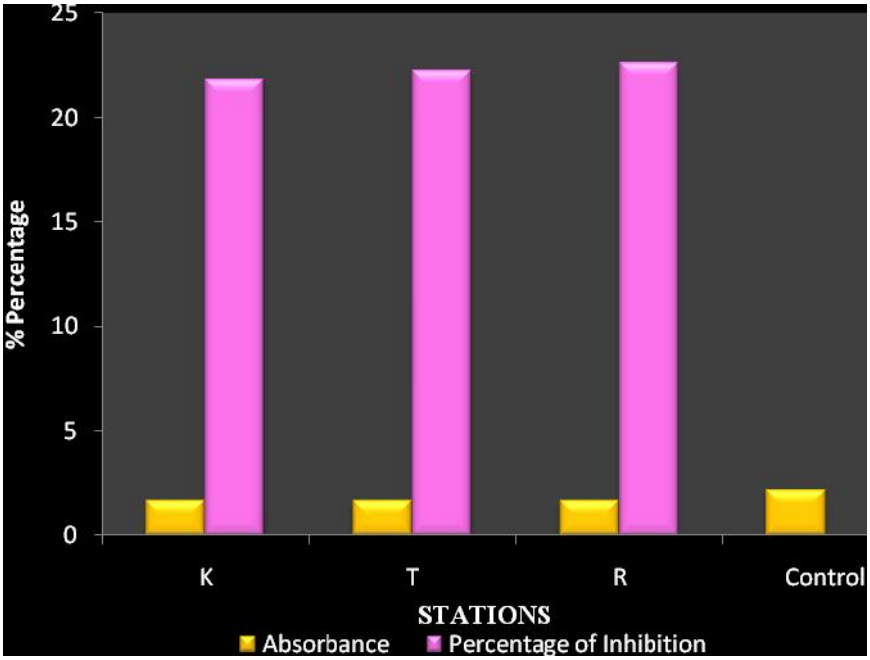
The observed results were tabulated and discussed on its activity basis with a pretext to each enzyme studied.

Catalase assay

The assay was performed and the results were calculated as in the form of free radical percentage exhibited by the crab and compared with a different location and it showed the precisely as same point and percentage of inhibition in all location of crab at approximately 22-23% (Table 1).

Table 1: Free radical inhibition percentage of the Crab *Portunus pelagicus.* (Kanyakumari (Cinna Muttam) (K), Thoothukudi (Therespuram) (T), Rameshwaram (R))

Sample	Absorbance	Percentage of Inhibition
K	1.715	21.83226983
T	1.705	22.28805834
R	1.698	22.6071103
Control	2.194	



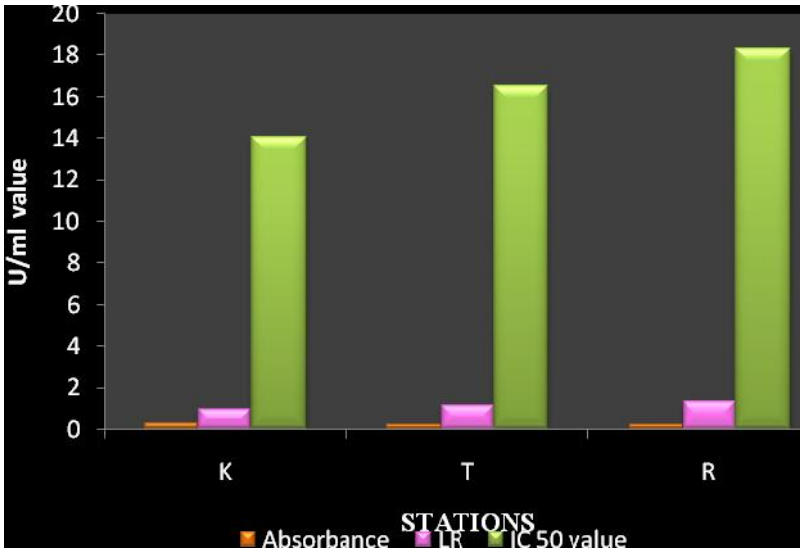
Superoxide dimutase assay

The SOD was calculated on the basis of standard preparation and the test values were recorded on its absorbance value and from the IC50 value were calculated.

The IC50 value of different location was calculated by linearization of the value and by regression graph analysis. From the observed results the Kanyakumari showed 14.07535 U/ml, Thoothukudi showed 16.53991 U/ml and Rameshwaram recorded 18.33313 U/ml respectively (Table 2).

Table 2 : SOD activity of *Portunus pelagicus* crab of different locations

Sample		Absorbance	LR	IC50 value
Standard	S1	0.252	1	
	S2	0.217	1.16129	
	S3	0.164	1.536585	
	S4	0.115	2.191304	
	S5	0.089	2.831461	
	S6	0.071	3.549296	
	S7	0.059	4.271186	
K		0.254	0.992126	14.07535
T		0.214	1.17757	16.53991
R		0.192	1.3125	18.33313



Discussion

The crab samples collected from fishing harbours and beaches are exposed to constantly fluctuating and extreme conditions, for example, temperature, salinity, pH, and heavy metal pollution, causing intermittent intracellular oxidative stress conditions developed by the accumulation of reactive oxygen species (ROS). In order to prevent destructive changes resulting from the potentially reactive O_2^- anions, cells possess SOD and catalase as a defense system against these reactive ions. Different types of SOD enzyme have been discovered till date such as cytocolic SOD and mitochondrial SOD. Cytosolic SOD contains Cu/Zn and mitochondrial SOD contains Mn (Kim and Lee, 1997). SOD and CAT may be induced in mild oxidative stress conditions as a compensatory response. However, oxidative damage might occur when the reactive oxygen species are produced to a higher level, thereby suppressing the activities of the antioxidant enzymes (Zhang *et al.*, 2004). SOD and CAT system provides the first line of defense against oxidative damage at a cellular level (Walters *et al.*, 2016). Seasonal variations in oxidative stress responses in mud crabs (*Scylla serrata*) was observed with a noted increase in the antioxidant defense during summer, which might be related with the changes in the temperature (Paital and Chainy, 2013). Temperature fluctuations alter the metabolic rates as well as oxygen consumption, thereby leading to oxidative stress (Walters *et al.*, 2016).

The free radical inhibition percentage of catalase activity of the blue swimming Crab *P. pelagicus*. The highest percentage inhibition was observed in crab isolated from Rameshwaram with 22.6%, followed by 22.28% inhibition for Thoothukudi and 21.83% inhibition for Kanyakumari. The Super Oxide Dismutase content was found to be the highest in the crab sample of Rameshwaram with 18.33 U/ml of sample. Overall, the stress enzymes were found to be

elevated in the crab samples isolated from Rameshwaram. The differences in the stress enzyme levels of the blue swimming crab *P. pelagicus* suggest that abiotic factors such as temperature, salinity and dissolved oxygen content may influence the levels of antioxidant enzymes and, thereby the oxidative stress status in a tissue and sex specific manner.

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