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Partial purification of peroxidase from marine algae

L. Jeyanthi Rebecca*, S. Sharmila and E. Kowsalya

Department of Industrial Biotechnology, Bharath University, 173, Agharam Road, Selaiyur, Chennai-600 073

ABSTRACT

Peroxidases are known for their role in scavenging free radicals and hence it is an efficient antioxidant. Seaweeds are a rich source of industrial enzymes and in the present work it was used to study the presence of peroxidases. The peroxidases were isolated and partially purified from Sargassum sp and Enteromorpha sp collected from coastal areas of Chennai, Puducherry, Tuticorin and Kanyakumari. The peroxidase activity was more in Sargassum sp collected from Kanyakumari when compared to the sample collected from Tuticorin coast. Similarly the enzyme activity was slightly more in Enteromorpha sp samples collected from Puducherry.

Keywords: seaweed, *Sargassum*, *Enteromorpha*, peroxidase activity

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INTRODUCTION

The term isozyme was coined by Market and Moller (1959) [1] to differentiate between molecular forms of proteins that had same enzymatic specificity [2]. Isozymes are a powerful tool for gene variability within and between populations of plants and animals. They are also used to select desirable genotypes within the population. The percentage of variation shown by isozyme zymograms was more when compared to the percentage of morphological variation. Multiple forms of esterase, amylase, β-glucosidase, sucrase, cellulose, protease and alkaline phosphatase have been identified from *Aspergillus oryzae* [3].

The enzyme peroxidase exists in isoforms in many plant species. They have been used to study the effect of viral infection on host plant [4]. Hence peroxidases are involved in plant defense and are expressed during cell damage. A lot of work has been done to study the evolutionary pattern of plants using isozymes in the form of protein profiling and to ascertain the variation in different traits. Seaweeds have low amounts of electrophoretically detectable genetic variation when compared to plants [5].

Seaweed grows abundantly along the coasts of Tamil Nadu, Gujarat and around Lakshadweep and Andaman and Nicobar islands. They are commercially important and have wide applications in the pharmaceutical, cosmetic and food industry. Lipases and proteases are becoming increasingly important in high-value applications in the medicinal industry and the production of a variety of chemicals and drugs [6-12]. They have been used as adsorbent in the bioremediation of heavy metals from effluents [13].

Catalase-peroxidase has a protective role against environmental H_2O_2 generated by algae or bacteria in the ecosystem. This protective role is most apparent at high cell density of the cyanobacterium. The residual H_2O_2–scavenging activity in the ΔkatG mutant was a light-dependant peroxidase activity. However, neither glutathione peroxidase nor ascorbate peroxidase accounted for a significant part of this H_2O_2-scavenging activity [14]. Seaweed

crude extracts acts to protect against acetaminophen-induced lipid peroxidation through their free radical scavenging property [14]. The present study was undertaken to study the variation in peroxidase activity from *Sargassum sp* and *Enteromorpha sp*.

MATERIALS AND METHODS

Collection of Samples

Seaweeds were collected from Kanyakumari, Tuticorin, Covelong, Chennai and Pulicat coastal regions of Tamil Nadu. They were later identified by Dr. Lita Sundar, Assistant Professor, Department of Coastal Resource Management, Madras Christian College. Tambaram. The samples were identified as a species of *Sargassam* and *Enteromorpha.*

Preparation of Samples

The samples were washed thoroughly and dried in the sunlight for 24 hours to 48 hours depending on the intensity of sunlight.

Protein Estimation

First 0.5 gm of each seaweed samples was taken and ground with 3 ml of NaOH drop by drop using a pestle and mortar. Then it was centrifuged for 15 minutes at 10,000 rpm. After centrifugation, the supernatant was taken out and stored. Protein was estimated by Bradford method.

Enzyme activity of Peroxidase

Guaicol is used as substrate for the assay of proxidase. Guaicol + $H_2O_2 \xrightarrow{POD}$ Oxidised guaicol + 2 H_2O. 1g of algal sample was mixed with 3ml of 0.1 M phosphate buffer pH 7.0 and ground in a pre-cooled pestle and mortar. The homogenate was centrifuged at 18,000 rpm at 5ºC for 15 minutes. The supernatent was used as enzyme source within 2-4 hours. 3ml buffer solution, 0.05ml guaicol solution, 0.1 ml enzyme extract and 0.03ml hydrogen peroxidase solution was taken in a cuvette. It was mixed well and the absorbance was measured using a spectrophotometer at 436 nm. A stopwatch was started after the absorbance reached 0.05 and the time taken in minutes for the absorbance (Δt) to increase 0.1 was noted down. Since the extinction co-efficient of guaicol dehydrogenation product at 436nm under the condition specified is 6.31 per micromole, the enzyme activity per litre of extract is calculated as below:

Enzyme activity (units/litre) – $\frac{3.18 \times 0.1 \times 1000}{6.39 \times 1 \times \Delta t \times 0.1} = \frac{500}{\Delta t}$.

Partial purification of peroxidase

The crude enzyme sample was mixed with ammonium sulphate to a saturation level of 75%. Then it was dialyzed overnight. The dialyzed sample was used as the enzyme solution for enzyme activity estimation.

RESULTS AND DISCUSSION

The seaweed samples were collected form coastal areas of Kanyakumari, Tuticorin, Covelong and Puducherry. They were identified as *Sargassum sp* and *Enteromorpha sp*. The partially purified samples were used after dialysis to estimate the peroxidase activity (Tables 1, 2). There were very slight variations in enzyme activity. However the determination of isozyme pattern using electrophoresis will shed more light on the variation between the samples.

Table 1 Peroxidase activity of *Sargassum sp*

Location	Sample	Volume (ml)	Total protein (µg)	Total enzyme (pkat)	Specific activity (pkat µg protein^{-1})
Kanyakumari	Crude extract	4	5000	18.93	3.8×10^{-3}
	Dialysed	0.5	305	30.67	10.1×10^{-2}
Tuticorin	Crude extract	4	5040	17.85	3.5×10^{-3}
	Dialysed	0.5	305	21.83	7.2×10^{-2}

Table 2 Peroxidase activity of *Enteromorpha sp*

Location	Sample	Volume (ml)	Total protein (µg)	Total enzyme (pkat)	Specific activity (pkat µg protein^{-1})
Kanyakumari	Crude extract	4	4680	25	5.3×10^{-3}
	Dialysed	0.5	302.5	19.37	6.4×10^{-2}
Puducherry	Crude extract	4	4680	20.83	4.4×10^{-3}
	Dialysed	0.5	310	21	6.8×10^{-2}
Covelong	Crude extract	4	4960	27.027	5.4×10^{-3}
	Dialysed	0.5	287.5	18.72	6.5×10^{-2}

CONCLUSION

Isoenzyme analysis with respect to peroxidase was carried out to find out the difference in enzyme activity between two samples of *Sargassum sp*. and three samples of *Enteromorpha sp* collected from different coastal areas of Tamil Nadu. There was a slight difference in enzyme activity between both the species. Further work on the isozyme pattern has to be carried out using electrophoresis.

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