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Research Article

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Isolation and partial purification of lipase and protease from marine algae

Shilpak Dilip Bele, Swapnil Suresh Patil, S. Sharmila and L. Jeyanthi Rebecca*

Department of Industrial Biotechnology, Bharath University, Agharam Road, Selaiyur, Chennai

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ABSTRACT

Lipases are a group of enzymes widely used in different industries as they catalyse the hydrolysis of the triacylgylcerols into its simpler compounds. They catalyze the process of transesterification which helps in the production of bio-fuel. These enzymes have a wide range of industrial applications. Protease is an essential constituent of all forms of life on earth including fungi, plants, prokaryotes and animals. They have a wide range of applications in various fields. It is well known that proteases hydrolyze peptide bonds of proteins and they differ in their substrate specificity and ability to hydrolyze various peptide bonds. The present study was carried out to partially purify protease and lipase from various varieties of seaweeds like Enteromorpha intestinalis, Enteromorpha clathrata, Gracilaria edulis, Ulva lactuca collected from the coastal area of Covelong and Pulicat Lake, Chennai, Tamilnadu. The protein content was estimated using Bradford method. The results indicate that the enzyme activity differs from species to species.

Keywords: Lipases, protease, seaweeds, transesterification, partial purification.

INTRODUCTION

Lipase**,** a subclass of esterases is any of a group of fat-splitting enzymes found in the blood, gastric juices, pancreatic secretions, intestinal juices, and adipose tissues [1, 2]. Lipases possess the ability to hydrolyze fats in the digestive tract but also act as a biocatalyst in carrying out acylation and deacylation of many unnatural substrates. They have been isolated and characterized from a wide range of sources [1-6]. They have a wide range of industrial applications [7, 8]. Certain pancreatic lipase inhibitors have been identified from seaweeds [9]. Esterases which can hydrolyze triglycerides at the water/oil boundary are termed lipases or, more systematically, triacylglycerol hydrolases [EC 3.1.1.3]. Lipases are stable in the terms of reactivity and surrounding environment and are obtained from plants, animals and different microbial flora.

Proteases are essential components of cells catalyzing processes ranging from photoacclimation and nutrient acquisition to development and stress responses. Alkaline proteases are of medical importance [10]. They have elastolytic and keratinolytic activity and hence can be used in leather-processing industries. Proteases find their use in the dehairing and in the processing of waste feathers [11, 12]. It has been used in the detergent industry and a bleach stable alkaline protease for detergent formulation from *Bacillus sp* is reported [13, 14, 15]. Thermostable alkaline protease is used to improve the processing capacity for the recovery of silver from used X-ray film [16, 17]. There are reports on the isolation of protease from plant leaves and marine waste like fish scales, crab and prawn shells [18-21].

The present study was carried out to isolate and purify protease and lipase from four species of marine algae.

EXPERIMENTAL SECTION

Collection of sample:
Samples were collected from two different places: Pulicat Lake and Covelong beach along the Bay of Bengal.

Identification of sample:
The collected samples were identified at Department of Marine Studies and Coastal Resource Management, MCC, Tambaram, Chennai. The samples from Pulicat Lake were identified as *Enteromorpha intestinalis, Enteromorpha clathrata, Ulva lactuca, Gracilaria edulis* and the sample from Covelong beach was identified as *Enteromorpha intestinalis*.

Processing of sample:
The samples were washed with fresh water thoroughly. Later they were kept under shade for drying. The sample was powdered and kept in the vessel for storage.

Estimation of protein content:
The protein content of the samples was estimated using Bradford method. The absorbance was taken at 590 nm in colorimeter [22].

Protein precipitation using Ammonium sulphate:
The samples were centrifuged at 10,000 rpm for 10 minutes. The precipitate was collected and dissolved in TCA buffer.

Partial purification by Sephadex G-25 column- Size exclusion chromatography:
The crude sample was passed through Sephadex G-25 column for desalting and for partial purification.

Plate Assay:
LB Agar plates were prepared and 1% (v/v) Tween 20 was added after autoclaving. The presence of white clear crystal around the enzyme well confirmed the presence of lipase activity. Casein agar media was prepared for protease assay. The well was made after the solidification of media and the partially purified enzyme was loaded. The wells were flooded with 10% Tannic acid and incubated for 24 hrs.

Lipase Activity:
Lipase activity was determined by titrometry method using two substrates castor oil emulsion and sunflower oil emulsion. The emulsion was prepared by mixing 25 mL of the oil and 75 mL of 7% Arabic gum solution in a homogenizer for 2 min. The reaction mixture containing 5 mL of the oil emulsion, 4 mL of 50 mM Tris-HCl buffer, pH 7.5, 1 mL of 110 mM $CaCl_2$ and 1 mL of partially purified enzyme solution was incubated at 37ºC for 30 min under orbital shaking at 200 rpm. The reaction was immediately stopped after the incubation period by the addition of 15 mL chloroform:ethanol mixture (2:1 v/v), and the released free fatty acids were titrated with 50 mM NaOH. The amount of NaOH was noted [23]. The lipase activity was calculated using the following formula.

Lipase activity= (vol. of alkali consumed * strength of alkali)/ (Wt. of sample in g * time)

Protease activity:
About 0.5 ml of enzyme extract was mixed with 0.5 ml casein. It was left for 1 hr at 37°C. Further 1 ml of 10% TCA was added. This mixture was centrifuged at 3000 rpm for 10 min and the supernatant was collected. About 0.5 ml of supernatant was mixed with Na_2CO_3 and NaOH and 0.75 ml of Folin Phenol. This mixture was shaken well and OD was taken at 650 nm. One protease unit is defined as the amount of enzyme that releases 0.5 µg/ml/min tyrosine. The amount of tyrosine was obtained from tyrosine standard curve.

RESULTS AND DISCUSSION

Two enzymes of industrial application was isolated and purified from marine algae. Their protein content is tabulated in Table 1. After ammonium sulphate precipitation, the protein content was more in *Enteromorpha* species. Crude samples of *G.edulis and E.intestinalis* showed highest protein concentration when compared to all other species. Protein content of the crude extract was found to be 179µg/ml and lowest was shown by *U.lactuca* i.e. 128µg/ml. The protein content of the partially purified samples after ammonium sulphate precipitation was also found. Protein concentration was found out to be 112µg/ml with *E.intestinalis* while in *U.lactuca* it was 82µg/ml.

The lipase activity was estimated using Titrometric method using two different substrates sunflower oil and castor oil. Table 2 shows the lipase activity of the different algal species. There was very slight difference in the lipase activity in among all the three species. The presence of lipase was initially detected by plate assay method. Among all the samples tested the activity of *E.intestinalis* from Covelong beach was observed to be the most active one. It was having the highest lipase activity among all i.e. 0.123meq/min/g with castor oil as a substrate Table 3.

Table 1- Protein content of samples

Sample	Protein content (µg/ml)	
	Crude extract	Ammonium sulphate precipitation
E. intestinalis (Pulicat)	169	107
E. intestinalis (Kovalam)	179	112
E. clathrata	148	98
U. lactuca	128	82
G. edulis	179	102

Table 2- Lipase activity using sunflower oil as substrate

S.No.	Name of sample	Initial pH	After addition of enzyme	Volume of NaOH (ml)	Time (min)	Final pH	Wt of pellet (g)	Activity meq/min/g
1.	*E.intestinalis*(p)	7.02	3.6	10.5	12.52	7.20	0.73	0.115
2.	*E.intestinalis*(k)	7.10	3.58	10.9	13	7.00	0.68	0.122
3.	*E.clathrata*	7.06	3.89	9.1	10.85	7.10	0.70	0.119
4.	*U.lactuca*	7.00	3.66	8.9	10.61	7.04	0.69	0.121
5.	*G.edulis*	7.2	3.90	8.1	9.66	7.10	0.79	0.106

Table 3- Lipase activity using castor oil as substrate

S. No.	Name of sample	Initial pH	After addition of enzyme	Volume of NaOH (ml)	Time (min)	Final pH	Wt of pellet (g)	Activity meq/min/g
1.	*E.intestinalis* (p)	7.02	4.25	9.6	11.44	7.00	0.73	0.114
2.	*E.intestinalis*(k)	7.10	4.46	6.86	8.18	7.13	0.68	0.123
3.	*E.clathrata*	7.06	4.32	7.1	8.46	7.04	0.70	0.119
4.	*U.lactuca*	7.00	4.12	7.5	9.94	7.10	0.69	0.109
5.	*G.edulis*	7.2	4.7	6.2	7.39	7.08	0.79	0.106

There was no significant difference in the lipase activity using the two substrates. However a study using different concentrations of substrate is required.

Table 4- Protease activity

Species Name	Place	Protease activity (units/mg)
E. intestinalis	Pulicat	10.24
E. intestinalis	Kovalam	10.8
E. clathrata	Pulicat	7.36
U. lactuca	Pulicat	6.4
G. edulis	Pulicat	8.8

The protease activity was initially confirmed by plate assay method.Protease activity was found to vary with different species. *E. intestinalis* from Covelong showed highest activity Table 4.

CONCLUSION

Lipases and proteases are becoming increasingly important in high-value applications in the medicinal industry and the production of variety chemicals and drugs. The current study shows that seaweeds can be used as good source of protease and lipase enzyme. Apart from these enzymes, seaweeds can also be screened for various other industrially important enzymes which may hold application in various fields. The enzyme activity and protein content varied with species and geographical area. *E. intestinalis* was found to show the highest activity of both the enzymes. We are currently extending our work in the characterization of the enzymes.

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