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Comparative Study on Methods of Extraction of Protease from Marine Algae.

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ABSTRACT

Protease is essential constituents of all forms of life on earth including fungi, plants, prokaryotes and animals. 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 compare two methods of protease extraction namely, protein precipitation using ammonium sulphate and two phase extraction using PEG-4000 from seaweeds. The species studied were *Enteromorpha intestinalis, Enteromorpha clathrata, Ulva lactuca, Gracilaria edulis*. The protein content was estimated using Bradford method. The enzyme assay was estimated spectrophotometrically as well as by plate assay method. The results indicate that protease activity differs with species of seaweeds.

Key words: seaweed, protease activity, ammonium sulphate, PEG

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INTRODUCTION

Seaweeds are algae that live in sea or in brackish water. Three groups of seaweeds are recognized, according to their pigments that absorb light of particular wavelengths and give them characteristic colour of green, brown or red [1]. Nowadays, we use extracts from certain seaweeds as stabilizers, gelling agents or emulsifiers, in thousands of everyday products from pet food to dental moulds [2]. Agar, the jelly found in seaweeds, is irreplaceable as a medium on which to culture fungi and bacteria for medical testing and research in microbiology [3-4].

Proteases are essential components of cells particulate in processes ranging from photoacclimation and nutrient acquisition to development and stress responses. Alkaline proteases are also used for developing products of medical importance [5]. Alkaline proteases with elastolytic and keratinolytic activity can be used in leather-processing industries. Proteases find their use in the soaking, dehairing, processing waste feathers [6] [7]. A novel process for stabilizing detergent enzymes against oxidation has been provided and beach stable alkaline protease for detergent formulation from *Bacillus sp* is reported [8-10]. Thermostable alkaline protease was used to improve the processing capacity for the recovery of silver from used X-ray film [11-12].

There are reports on the isolation of protease from plant leaves and marine waste like fish scales, crab and prawn shells [13-16]. Kudrya and Simonenko exploited the elastolytic activity of B. subtilis 316M for the preparation of elastoterase, which was applied for the treatment of burns [17]. The present study was carried out to compare two methods of extraction of protease enzyme from marine algae namely, ammonium sulphate precipitation and two phase extraction using PEG-4000.

MATERIALS AND METHODS

Collection and preparation of samples

Different samples of marine algae were collected from Covelong (Eastern coast of Tamil Nadu, India) and from Pulicat lake (North eastern coast of Tamil Nadu, India).

Identification of sample

The collected samples were identified at Department of Marine Studies and Coastal Resource Management, MCC, Tambaram, Chennai. The species from Covelong was identified as: *Enteromorpha intestinalis.* The species from Pulicat was identified as: *Enteromorpha clathrata, Enteromorpha intestinalis, Ulva lactuca, Gracilaria edulis.* The collected samples were thoroughly rinsed with water to remove anyassociated debris. The algae after rinsing were dried carefully in shade under room temperature for 10 days and were crushed to powder.

Extraction of Enzyme

The powdered samples were soaked in 10% TCA over night. Further it was centrifuged at 10,000 rpm for 10 min. The supernatant was separated for further purification.

Estimation of protein content by Bradford Method

The protein concentration was estimated by Bradford method, using BSA as a standard. The colour change was measured at 595 nm [18].

Protein precipitation using Ammonium sulphate

The supernatant was fractioned by 70% ammonium sulphate precipitation. The protein pellet obtained after precipitation was resuspended in 10% TCA.

Two phase extraction using PEG-4000

The crude sample was centrifuged and the supernatant was collected. About 0.75 gm of sodium sulphate was added to 10 ml of sample. About 3ml of 50% PEG-4000 was added and checked for two phase separation. It was allowed to settle and the two phases were pipetted out using micropipette.

Partial purification by Sephadex column

Sephadex G-25 was used to pack the column. The solution was fed to sephadex column for desalting and partial purification.

Plate Assay

Casein agar media was prepared. 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.

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 supernatant was collected. 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.

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RESULTS AND DISCUSSION

The present study comprises four different species namely, *Enteromorpha clathrata, Enteromorpha intestinalis, Ulva lactuca, Gracilaria edulis* collected from Covelong and Pulikat Lake for extracting the enzyme.

Table 1: Protein content of crude samples

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

Protein precipitation using Ammonium sulphate

Enteromorpha sp had more protein when compared to *Gracilaria edulis* and *Ulva lactuca* (Table 1). The protein content of seaweeds belonging to the same species varied from place to place [16]. The enzyme actitity was initially verified using plate assay method which showed zone of inhibition indicating the presence of protease. The protease activity after ammonium sulphate precipitation is listed in Table 2. *E. intestinalis* from Kovalam showed a maximum activity of 10.8 units/mg of protein and *U. lactuca* showed least activity of 6.4 units/mg of protein. *Gracilaria corticata* showed maximum enzyme activity of 7.53 units/mg of protein and *Ulva sp* showed least enzyme activity of 5.88 units/mg of protein [3]. In the present study, *U. lactuca* showed the least enzyme activity.

Table 2: Protease activity after ammonium sulphate precipitation

Species Name	Place	Protease activity (units/mg of protein)
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

Protein precipitation using two phase separation using PEG-4000

The result of the titration of PEG-4000 against sodium sulphate is shown in Table 3. From the results it was concluded that 50% of PEG was ideal for the protease precipitation. The protein content after PEG-4000 precipitation is shown in Table 4.

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Table 3: Titration of PEG-4000 against sodium sulphate

S.No.	% PEG	%Na_2SO_4	Volume of PEG (ml)	Volume of Na_2SO_4 (ml)	Total Volume (ml)	Weight of PEG (g)	Weight of Na_2SO_4 (g)
1	10%	25%	5ml	2.5	7.5	0.5	0.723
2	20%	25%	5ml	1.9	6.9	1.0	0.475
3	30%	25%	5ml	1.6	6.6	1.5	0.400
4	40%	25%	5ml	1.4	6.4	2.0	0.35
5	50%	25%	5ml	1.3	6.3	2.5	0.325

Table 4: Protein precipitation using PEG-4000 and citrate buffer

Samples	Protein content (mg/ml)
E. intestinalis (Kovalam)	132
E. intestinalis (Pulicat)	127

Table 5: Protease activity after precipitation with PEG-4000

Samples	Protease activity (units/mg of protein)
E. intestinalis (Kovalam)	10.56
E. intestinalis (Pulicat)	10.24

The protease was extracted from only two samples namely, *E. intestinalis* (Pulicat) and *E. intestinalis* (Kovalam) using PEG-4000 as it showed a higher amount of protein content. The protease activity after precipitation with PEG-4000 is shown in Table 5. Protease activity was found to vary with different species. *E. intestinalis* from Covelong showed highest activity when compared to other species.

CONCLUSION

The current study shows that seaweeds can be used as good source of protease enzyme. Apart from protease, seaweeds can also be screened for various other industrially important enzymes which may hold application in various fields. The protease activity varied with species and geographical area. *E. intestinalis* was found to show the highest activity. In the current study, it was found that ammonium sulphate method was the most suitable for protein extraction.

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