Research Article

Isolation and Characterization of Protease from Marine Algae

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ABSTRACT

A protease is a member of a very large group of enzymes that have various functions in the body. Proteases break the long chainlike molecules of proteins into shorter fragments. Proteases are found in plants, animals, including microorganisms such as fungi, bacteria and yeast. Protease is considered to be an important industrial enzyme accounting for about 60% worldwide sale of enzymes. The influence of parameters such as temperature, pH were evaluated.

Keywords: Algae, Electrophoresis, Protein content, Protease, purification.

INTRODUCTION

The ocean is considered to be the repository of many unexploited natural resources. Protease hydrolyzes the peptide bonds and is also termed as proteinase.[1] Protease constitutes one of the most important groups of industrial group having application in different sectors of industries. Protease is widely being used in waste management, silver recovery from X-ray films, leather industry, and detergent industry.[2-4]

Proteases have found applications in molecular biology research as well.[5] A protease from Bacillus amyloliquefaciens may be used to promote flavor production in cheddar cheese.[6] Proteases are also being used in meat processing, dairy, digestive aid[7] Protease has found an irreplaceable role in silk industry.[8,9]

Reports are available on protease production by fungal species belonging to *Aspergillus* genera.[10] There are reports on the isolation of protease from plant leaves and marine waste like fish scales, crab and prawn shells.[11-14]

MATERIALS AND METHODS

Estimation of protein content by Bradford Method

The protein concentration was measured by the Bradford method, using BSA as a standard. Dilute the Bradford reagent and add the sample to the diluted reagent. The colour was changed to dark blue. The absorbance was taken at 590 nm.[15]

Protein precipitation using Ammonium sulphate

Ammonium sulphate was used to precipitate the protein. Appropriate amount of ammonium sulphate is added to the supernatant and centrifuged for 10 mins at 10000 rpm. The pellets were resuspended in suitable buffer.

Partial purification by Sephadex column

Sephadex was used to pack the column. The solution was fed to the column for further purification.

Protease activity

0.5 ml of partially purified enzyme was mixed with 0.5 ml casein. The reaction mixture was incubated for 1 hr at 37°C and stopped by addition of 1 ml of 10% TCA. The mixture was centrifuged and supernatant was collected. 0.5 ml of supernatant was mixed with Na2CO3 and NaOH and Folin Phenol. This mixture was shaken well and OD was taken at 650nm. One protease unit is defined as the amount of enzyme that releases 0.5 µg/ml/min tyrosine. The protease activity was measured spectrophotometrically.

Effect of pH and Temperature

The optimum pH of the enzyme was determined using buffer at varying pH ranges of 3-12. The effect of temperature on the protease activity was studied by incubating the reaction mixture at different temperature ranging from 20-90°C.

Sodium Dodecyl Sulphate-Polyacrylamide gel electrophoresis (SDS-PAGE)

SDS-Polyacrylamide gel electrophoresis was performed on slab gel with separating and stacking gels by the method of (Laemmli, 1970).[16]

RESULTS AND DISCUSSION

The present study was carried out to isolate and characterize protease from algae. The protein content of all the samples is shown in Table 1.

Table 3 and table 4 clearly show that parameters like temperature and pH affect the activity of enzyme. Maximum species showed highest activity at 40°C. The optimum pH was checked for all the samples at varied pH range. The optimum pH range was observed between 7-7.5 depending on species, although the maximum activity was seen at pH 7.2.

It can be observed from the above result that the isolated enzyme from different species had molecular weight

within the range of 30-45 kDa. The bands were compared with the standard bacterial protease run alongside.

Table 1: Protein estimation of crude and purified samples

Species Name	Protein content of crude extract (µg/ml)	Protein content (purified) (µg/ml)
Ulva lactuca (Puducherry)	92	82
Ulva fasciata (Puducherry)	97	82
Enteromorpha compressa (Puducherry)	86	77
Chaetomorpha antenna (Puducherry)	105	90
Ulva lactuca (Mahabalipuram)	92	77
Enteromorpha flexuosa(Mahabalipuram)	110	90

Table 1 shows the protein content of 6 species of seaweed. The highest protein content was seen in *Enteromorpha flexuosa* (Mahabalipuram) and *Chaetomorpha antenna* (Puducherry) while lowest was found in *Ulva lactuca* (Mahabalipuram) and *Enteromorpha compressa* (Puducherry).

Table 2: Activity of protease using Casein as substrate

Species Name	Place	Protease activity (units/mg)
Ulva lactuca	Puducherry	7.33
Ulva fasciata	Puducherry	8.0
Enteromorpha compressa	Puducherry	6.74
Chaetomorpha antenna	Puducherry	9.4
Ulva lactuca	Mahabalipuram	6.55
Enteromorpha flexuosa	Mahabalipuram	9.6

The above Table shows *Enteromorpha flexuosa* showing the highest activity while *Ulva lactuca* (Mahabalipuram) showed the lowest.

Table 3: Effect of Temperature on Protease activity

Species	Temperature Range (°C)					
	20	30	40	50	60	70
Ulva lactuca	6.33	6.71	7.35	6.4	5.20	4.42
Ulva fasciata	6.92	7.31	8.0	7.37	5.32	3.9
Enteromorpha compressa	5.22	6.35	6.77	6.20	4.39	3.55
Chaetomorpha antenna	6.78	8.42	9.49	8.11	6.22	5.14
Ulva lactuca	4.52	5.91	6.61	5.33	4.59	4.38
Enteromorpha flexuosa	6.55	8.61	9.38	7.52	6.17	5.21

Table 4: Effect of pH on Protease activity

Species	pH range			
	6.5	7.0	7.5	8
Ulva lactuca	6.21	7.25	7.41	6.79
Ulva fasciata	6.42	8.0	8.22	7.66
Enteromorpha compressa	5.85	6.71	6.77	5.73
Chaetomorpha antenna	8.22	9.12	9.22	8.76
Ulva lactuca	5.73	6.43	6.47	5.95
Enteromorpha flexuosa	8.46	9.48	9.59	8.66

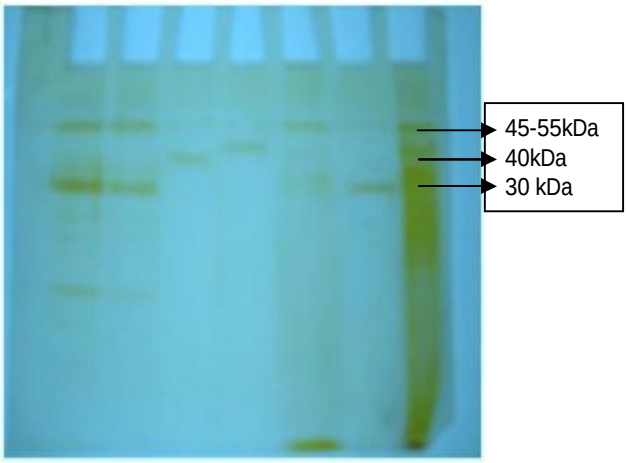

Figure 1: Estimation of molecular weight of algal protease using SDS-PAGE

Note: Lane 1 - Ulva lactuca (Puducherry); Lane 2 - Ulva fasciata (Puducherry); Lane 3 – Ulva lactuca (Mahabalipuram); Lane 4 – Enteromorpha compressa (Puducherry); Lane 5 – Chaetomorpha antenna (Puducherry); Lane 6 – Enteromorpha flexuosa (Mahabalipuram); Lane 7 – Standard bacterial Protease

CONCLUSION

The major aim of the study carried out was to investigate, characterize and purify the protease enzyme from various marine algae. The enzyme extracted from different marine algae showed positive results for protease assay confirming the presence of protease. The activity varied with different species and temperature and pH.

From this study, it is quite evident that marine algae are a valuable source of Protease. Moreover, they can also be screened for other industrially important enzymes.

REFERENCES

1. Barrett AJ, Mcdonald JK, Nomenclature: Protease, Proteinase and Peptidase. Biochem. J, 237, 1986, 935.
2. Ray A, Protease Enzyme- Potential Industrial Scope, Int. J. Technol, 2, 2012, 1–4.
3. Fujiwara N, Tsumiya T, Katada T, Hosobuchi T, Yamamoto K, Continuous Recovery Of Silver From Used X-Ray Films Using A Proteolytic Enzyme, Process Biochem, 24, 1989, 155–156.
4. Babu NKS, Lakshmi KD, Afr.J.Biotechnol., 4(7), 2005, 724-726.

5. János András Mótyán, Ferenc Tóth, József Tőzsér, Research Applications Of Proteolytic Enzymes In Molecular Biology, Biomolecules, 3, 2013, 923-942.

6. www1.Lsbu.Ac.Uk/Water/Enztech/Index.Html.

7. Chellapan S, C Jasmin, M Soorej, Basheer, KK Elayas, Sarita G. Bhat, M. Chandrasekharn, Production, Purification And Partial Characterization Of Novel Protease From Marine Engyodontium Album Btmfs 10 Under Solid State Fermentation, Process. Biochem., 41, 2006, 956-961.

8. Das Sumana, Mathummal Sudarshan, Ashoke Ranjan Thakur, Shaon Raychaudhuri, Degumming Of Raw Silk Fabric With Help Of Marine Extracellular Protease. American Journal of Biochemistry And Biotechnology, 9(1), 2013, 12-18.

9. Yang Cao, Bochu Wang, Biodegradation Of Silk Biomaterials, Int J Mol Sci., 10(4), 2009, 1514–1524.

10. Fan-Ching Y, IH Lin, Production Of Acid Protease Using Thin Stillage From A Rice, Spirit Distillery By Aspergillus Niger, Enzyme Microb. Technol., 23, 1998, 397-402.

11. L Jeyanthi Rebecca, S Sharmila, Merina Paul Das, F Abraham Samuel, Production and Analysis of Protease From *Aspergillus Niger* Using Fish Scales As Substrate, Journal Of Chemical And Pharmaceutical Research, 4(10), 2012, 4597-4600.

12. L Jeyanthi Rebecca, S Sharmila, Merina Paul Das, TV Rishikesh, S Ananda narasimhan, Production And Analysis Of Protease And Amylase From *Aspergillus Niger* Using Crab And Prawn Shell As Substrate, Journal Of Chemical And Pharmaceutical Research, 4(10), 2012, 4542-4544.

13. S Sharmila, L Jeyanthi Rebecca, Merina Paul Das, Md Saduzzaman, Isolation and Partial Purification Of Protease From Plant Leaves, Journal Of Chemical And Pharmaceutical Research, 4(8), 2012b, 3808-3812.

14. V Dhanalakshmi, L Jeyanthi Rebecca, G Revathi, S Sharmila, Evaluation Of Basic Nutritional Parameters Of Seaweeds In Coastal Tamilnadu, International Journal Of Biotechnology And Biochemistry, 6(6), 2010, 921-928.

15. Bradford M, A Rapid And Sensitive Method For The Quantization Of Microgram Quantities Of Protein Utilizing The Principle Of Protein-Dye Binding, Anal. Biochem., 72, 1976, 248-254.

16. Laemmli UK, Nature, 227, 1970, 680.

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