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Extraction of Crude Protease from Seaweed and its Preliminary Analysis

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Abstract: ***Enzymes are proteins that have catalytic functions indispensable to maintenance and activity of life. Enzymes are known to catalyze more than 5,000 biochemical reaction types. Algae are a good source of Protease enzyme which is used in meat tenerization and in textile industry. Totally, 4 species of marine algae showing protease activity were screened (Laurencia papillosa, Kappaphycus alvarezii, Padina gymnospora and Stoechospermum marginatum). Among the four, one species the best one (brown algae) was selected based on the zone of gelatin hydrolysis. The enzyme was extracted using 0.2M phosphate buffer (pH 7). Preliminary plate assay was carried out using casein, gelatin and skimmed milk medium and based on the zone formation due to protease hydrolysis, gelatin was selected because it shows better zone of clearance. Screening of four different algae against gelatin, skimmed milk, and casein study showed that the Padina gymnospora (substrate as gelatin) produced zone of inhibition 20mm, 20mm, 21mm, and 22mm respectively. SDS-PAGE was also carried out to characterize the protein, and the molecular size of the protein was estimated, bands were observed and compared with the protein marker run alongside. In the present study Padina gymnospora used gelatin to produce high yield.***

Keywords: Algae, Protease, Padina gymnospora, gelatin, enzyme

I. INTRODUCTION

Enzymes are proteins that have catalytic functions indispensable to maintenance and activity of life. At present, there are about 4,000 kinds of enzymes whose actions are well known. Enzymes are known to catalyze more than 5,000 biochemical reaction types. An enzyme's activity decreases markedly outside its optimal temperature and pH. (Williams HS,1904). Some enzymes are used commercially, for example in ation to break down proteins into smaller molecules, making the meat easier to chew.

Proteolytic enzyme, also called protease, proteinase, or peptidase, any of a group of enzymes that break the long chainlike molecules of proteins into shorter fragments and eventually into their components, amino acids. Proteolytic enzymes are present in bacteria , archea , certain types of algae, some viruses, and plants; they are most abundant, however, in animals.(Swapnil *et al.*, 2014). Proteases are found in all forms of microbes, plants and animal. They are found in some algae. (Rawlings N.D *et al.*,1993). Proteolytic enzymes are extremely important for the digestion of many foods. They also digest the cell walls of unwanted harmful organisms in the body and break down unwanted wastes such as toxins, cellular debris, and undigested proteins.

Algae can be referred to as plant-like organisms that are usually photosynthetic and aquatic, but do not have true roots, stems, leaves, vascular tissue and have simple reproductive structures. Most are microscopic, but some are quite large. They are known to be the source of many useful biological.

The objective of the current study is to obtain protease enzyme from marine algae. The biotechnology of microalgae has gained considerable importance in recent decades. Considering the enormous biodiversity of microalgae and with the development of sophisticated culture and screening techniques, micro-algal biotechnology can already meet the high demands of both the food and pharmaceutical industries.(Pulz *et al.*,, 2004).

II. MATERIALS AND METHODS

A. Collection and Processing of Marine algae

The marine algae namely *Laurencia papillosa, Kappaphycus alvarezii, Padina gymnospora* and *Stoechospermum marginatum* were been collected from Mandapam, Rameswaram, Tamil Nadu, India. The samples were then washed and rinsed with tap water and distilled water repeatedly. It was then kept in the refrigerator for experimental studies.

B. Extraction Process

10g of the sample was weighed and homogenized with 0.2M of phosphate buffer ($_p$H-7) (cooling condition) using mortar and pestle, it was then filtered. The filtrate was centrifuged at 10,000 rpm for 15 minutes. The supernatant was stored and used as enzyme source.

C. Screening Of Protease Production-Qualitative Assay (Preliminary Plate Assay)

Water agar medium (half strength) is to be supplemented with proteinous substrate (1% gelatin, casein and skimmed milk) for the assay of proteolytic enzyme was prepared and autoclaved at 121^0C for 15minutes. The plates were allowed to solidify and then, 5 wells (8mm diameter) were made by using a sterile cork borer. The 4 different volumes (25µl, 50µl, 75µl and 100µl) of the supernatant were loaded in the wells and 25µl of phosphate buffer was used as control. The plates were incubated for 24 hours at room temperature. After 24hours of incubation, the plates were flooded with (indicator) mercuric chloride ($HgCl_{2)}$ solution for 5 – 10 minutes. Protease production was visualized by a translucent zone around the wells. The zone of clearance were observed and measured.

D. Quantitative Assay For Protease Production-Enzyme Activity

Protease activity for the supernatant can be assayed according to the method of Mc Donald and Chen (1965).

An assay mixture was prepared by mixing 0.5ml of 1% gelatin with 0.5ml of cell free culture filtrate (enzyme source). The mixture was incubated at 37^0C for 60 minutes. 1ml of 10% trichloroacetic acid (TCA) was added to the reaction mixture. The reaction mixture was centrifuged at 10,000rpm for 15 minutes and the supernatant was collected. To 0.5ml of supernatant, 2.5ml of alkaline solution (2.9% Na_2C0_3 and 0.3N Na0H) and 0.75ml Folin phenol reagent (1ml of reagent diluted with 3ml of double distilled water before use was added and incubated at room temperature). After 20 minutes, the absorbance of the solution was measured at 650nm in a spectrophotometer. Simultaneously, control without the enzyme source was maintained. One unit of protease activity is defined as the amount of enzyme required to liberate 1µmol.tyrosine/ml/min. The protease enzyme was characterized by Sodium Dodecyl sulphate-Polyacrylamide gel electrophoresis (SDS-PAGE) by the method of (Laemmli, 1970).

III. RESULTS AND DISCUSSION:

A. Collection And Processing Of Marine Algae

The marine algae was been collected and the processing steps such as cleaning in tap water and distilled water were performed in order to remove the unwanted materials. The marine algae was been then made dried and then were packed in sterile polythene bags, labeled and stored which was needed for further studies.

Fig.1: Collected Marine algae

Stoechospermum marginatum

Laurencia papillosa

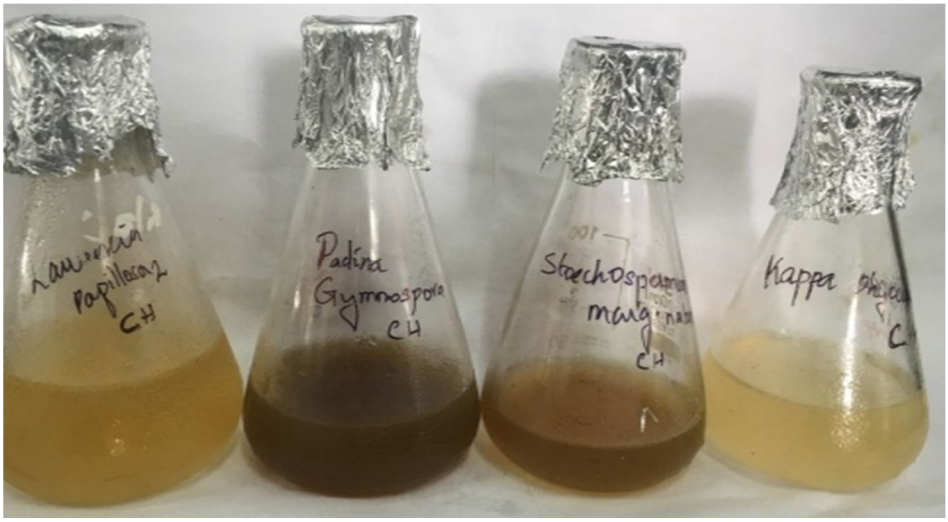
Kappaphycus alvarezii

Padina gymnospora

B. Extraction Process

The marine alga was homogenized with phosphate buffer (($_p$H-7) using mortar and pestle. The algae were homogenized since the cellular content will be released from the algae. Further, the filtrate was centrifuged so as to use the supernatant as the enzymatic source.

Fig.2: Extraction process of marine algae



C. Qualitative Assay (Preliminary Plate Assay)

The present study was conducted to produce and characterize protease from marine algae. Primary screening for protease producing algae was done on three different media (Gelatin, Casein and Skimmed milk), based on the zone formation due to protease hydrolysis. gelatin was selected because the zone of clearance was observed, whereas, skimmed milk exhibited zone of clearance in lower amount when compared to gelatin and casein did not exhibit the zone of clearance. Totally, four species of marine algae showing protease activity were screened (Laurencia papillosa, Kappaphycus alvarezii, Padina gymnospora and Stoechospermum marginatum). Among the four one species, the best one (brown algae) was selected based on the zone of gelatin hydrolysis.

Fig.3: Screening of marine alga for protease production-Gelatin (1%) as substrate

Protease is an industrially important enzyme having wider applications in pharmaceutical, leather, laundry, food and waste processing industries. Industrial enzyme production would be effective only if the organism and the target enzyme are capable of tolerating different variables of the production processes (Shilpa *et al.*, 2012). Primary screening for protease producing algae was done on three different media (Gelatin, Casein and Skimmed milk), based on the zone formation due to protease hydrolysis, gelatin was selected because it shows the zone of clearance, whereas, casein did not exhibit the zone of clearance.

IV. CONCLUSION

Protease play vital role in various industrial applications, the demand for protease is increasing day-by-day. Therefore it is expected that hyper active strains will emerge and that the enzymes produced by these new stains cold be used in different industries. In additions, it is imperative to identify new protease producing algae and perfect the fermentation technology in order to meet the ever-growing demand for this enzyme. The current study shows that seaweeds can be used as good source of Protease enzyme.

In the present work Padina gymnospora was found to be the producer of protease. Although, various microorganisms were reported to produce protease, there was no report on the production of protease by marine algae.

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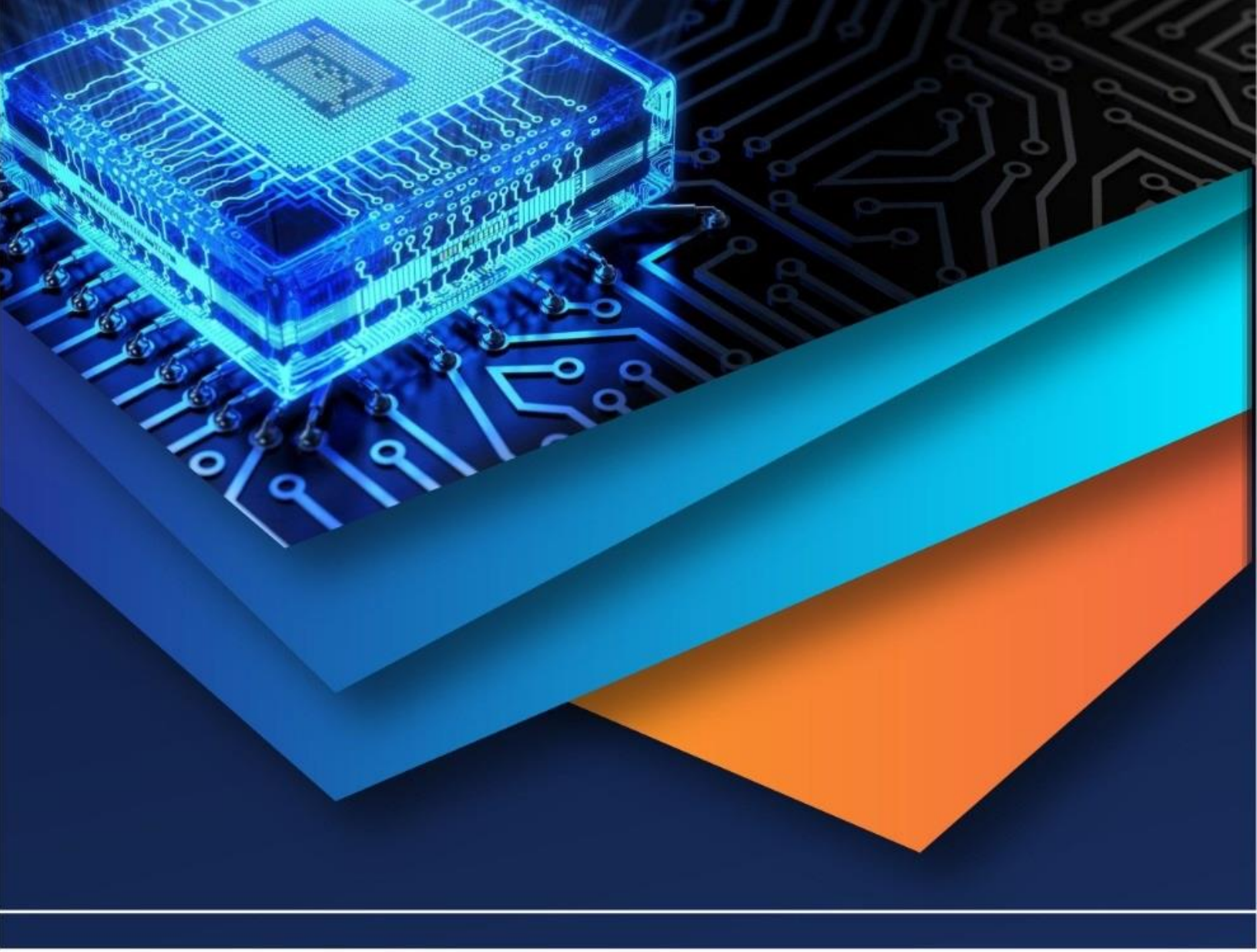

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