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Research Article

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Isolation and partial purification of Protease from plant leaves

S. Sharmila*, L. Jeyanthi Rebecca, Merina Paul Das and Md Saduzzaman

Department of Industrial Biotechnology, Bharath University, Chennai, India.

ABSTRACT

Protease occurs naturally in all organisms and is an essential constituent for all the existing live forms. Microorganisms such as bacteria and fungi and yeast are the main source of protease enzyme. They act as an important industrial enzyme occupying for about 60% of total enzyme market. In this study, protease was isolated from various leaves such as Coriandrum sativum, Nicotiana tobaccum, Murraya koenigii, and Moringa oleifera was partially purified. Then their specific activity and optimum pH were checked. Among the four plant species, the protease activity was found to be more in Nicotiana tobaccum (5.6 units/mg of protein) followed by Moringa oleifera (2.46 units/mg of protein), Murraya koenigii (2.02 units/mg of protein) and least in Coriandrum sativum (1.56 units/mg of protein). The optimum pH was found to be 7.2 for all the samples.

Keywords: Protease; *Coriandrum sativum; Nicotiana tobaccum; Murraya koenigii; Moringa oleifera;* Specific activity.

INTRODUCTION

Enzymes are well known biocatalyst that perform a multitude of chemical reaction and are commercially used in detergents, food, pharma, diagnostic and fine chemical industries [1].

Protease is an enzyme which is widely used in detergents, leather, waste management and silver recovery [2]. In Japan 1994, alkaline proteases sale were estimated at 1500 million yen. The sources of protease are enormous. *Bacillus sp.*, were found to be predominant and a rich source of alkaline proteases. Many of the fungi have also been reported to produce extracellular alkaline protease [3]. Among various proteases, bacterial proteases are more significant compared to with animal and fungal protease [4].

Microbial proteases were proposed as virulence factors in a variety of disease caused by microorganism. In bacteria serine and metallo proteases are the main classes of proteases. They are present in *Bacillus subtilis, B.amyloliquefaciens, Pseudomonas sp., Lysobacter enzymogene, E.coli* [5].

Medicinal seeds were also investigated for their kinetic activity of protease, since these seeds are used to treat various diseases [6]. Herbs play a major tool in the eastern style medicine known as Unani Hikmat. *Convalia ensiformis* beans are also used for the production of protease [7].

Many types of seaweed were identified for the production protease enzyme [8].

Plants are the rich source of protease enzyme. Greenberg and Winnick [9] listed eleven plant proteases. Reports on protease present in green leaves which do not produce latex are very few. In 1900, Loew [10] isolated protease from green leaf of tobacco. Many enzymes were present in plant cell wall [11]. With the exception of bromelin, pinguinain and solanain are obtained from latex of plants. These are obtained from fruits. Bromelin also occurs in green leaves of pine apple. Other plant proteases which have been studied in detail are those of seeds, both dormant and germinating.

In this study, dried leaves of *Nicotiana tobaccum*,and green leaves of *Murraya koenigii, Moringa oleifera* and *Coriandrum sativum* were selected to isolate the protease enzyme and to study their specific activity and optimum pH. The parameters of protease enzyme were also compared.

EXPERIMENTAL SECTION

Extraction of enzyme

The samples were homogenized using mortar and pestle with phosphate buffer pH 7.0. Then it was centrifuged at 10,000 rpm for 10 min. The supernatant was collected and was subjected to further purification.

Partial purification of enzyme

The protein from the crude enzyme was precipitated using 70% ammonium sulphate. Then it was fed into sephadex column for desalting.

Estimation of protein concentration

The protein concentrations of the samples were analyzed by Bradford method [12].

Protease activity

Partially purified protease was mixed with 0.5ml of casein and was left for 1 hr at 37^{o}C. Then 10%TCA was added to the solution. The mixture was centrifuged at 3000rpm for 10 min and the supernatant was collected. Then the supernatant was mixed with protease reagent and folins phenol. It was shaken well and the OD was checked for every 5 min at 650nm.

Estimation of pH

The optimum pH for the protease was checked out using phosphate buffer at 5.7, 6.2, 6.7 and 7.2.

RESULTS AND DISCUSSION

Protease plays a vital role in many pathological processes. Arthritis, tumor invasion and metastasis , infections and number of degenerative disease have been linked with proteolytic enzymes [13]. They have found extensive applications in bioremediation processes [14].

In this study, protease was isolated from various leaves such as *Coriandrum sativum, Nicotiana tobaccum, Murraya koenigii,* and *Moringa oleifera* was partially purified. After partial purification protein concentration was found out by Bradford method. Table.1 shows the protein concentration of the partially purified protease. Among these samples, protein concentration was more in *Moringa oleifera* (1.3 µg/ml) followed by *Nicotiana tobaccum* (1.09 µg/ml), *Coriandrum sativum* (1.05 µg/ml), and less in *Murraya koenigii* (0.95 µg/ml).

Table.1 Concentration of protein

S.No	Sample	Protein Concentration (µg/ml)
1	*Nicotiana tobaccum*	1.09
2	*Moringa oleifera*	1.3
3	*Murraya koenigii*	0.95
4	*Coriandrum sativum*	1.05

Table.2 Specific activity of protease

S.No	Sample	Specific activity (units/ mg of protein)
1	*Nicotiana tobaccum*	5.6
2	*Moringa oleifera*	4.27
3	*Murraya koenigii*	3.32
4	*Coriandrum sativum*	2.47

Table.2 evident that activity of protease was more in *Nicotiana tobaccum* (5.6 units/ mg of protein) followed by *Moringa oleifera* (4.27 units/ mg of protein), *Murraya koenigii* (3.32 units/ mg of protein) and less in *Coriandrum sativum* (2.47 units/ mg of protein).

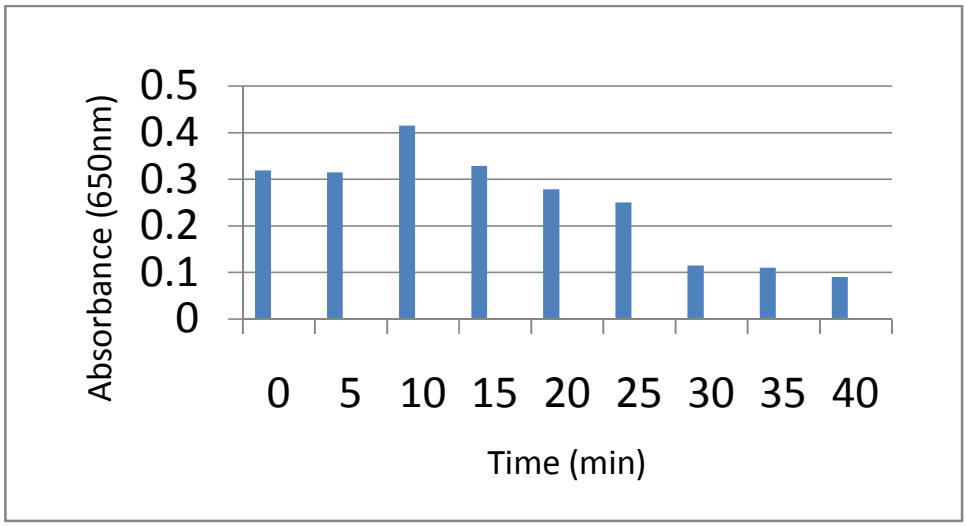

Fig.1Protease activity of *Coriandrum sativum*

Fig.1 shows that the protease activity of *Coriandrum sativum* was maximum at 10th min and for *Murraya koenigii* (Fig.2) activity was high at 15th min and suddenly dropped.

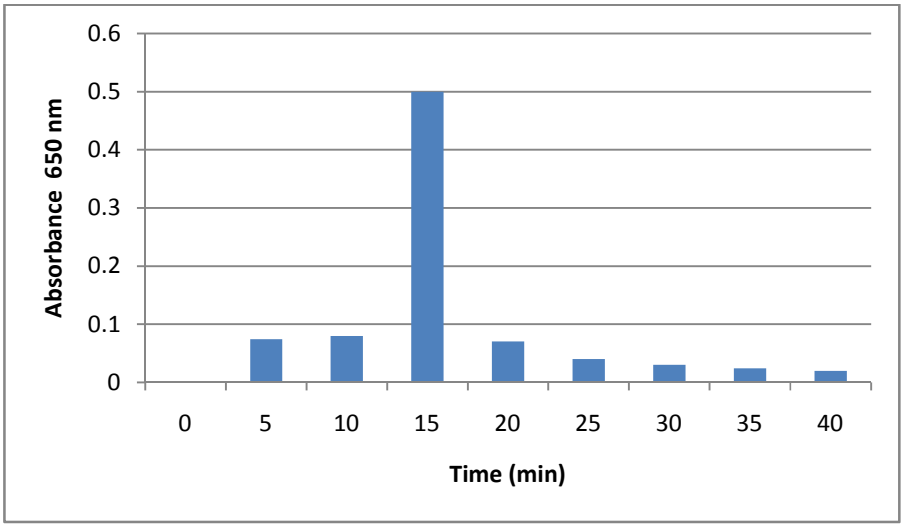

Fig .2 Protease activity of *Murraya koenigii*

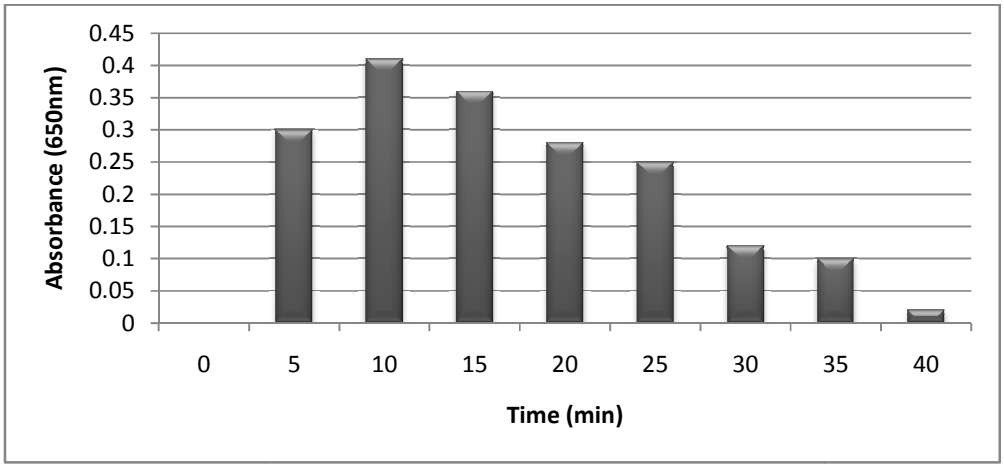

Fig.3 Protease activity of *Nicotiana tobacum*

After that *Nicotiana tobacum* (Fig.3) has highest specific activity at 10th min and for *Moringa oleifera* (Fig.4), it was found 15th min.

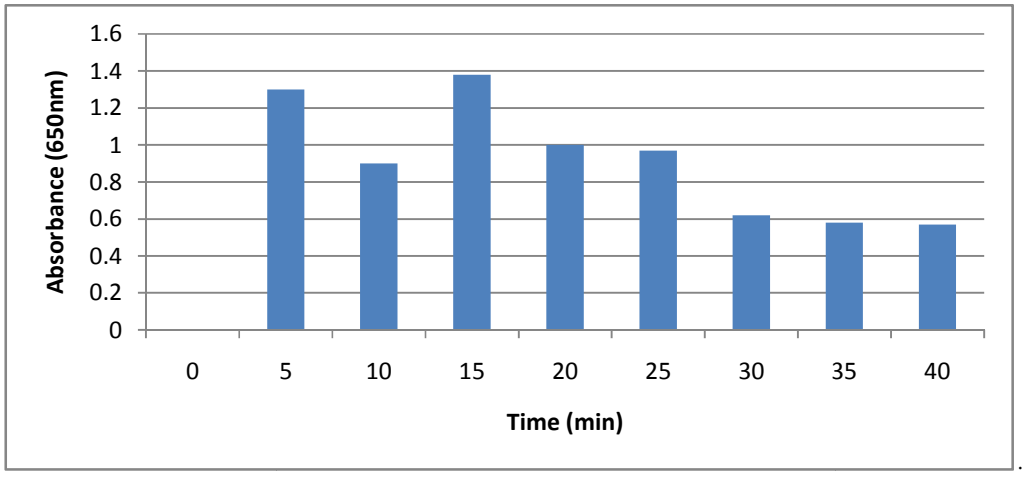

Fig.4 Protease activity of *Moringa oleifera*

Estimation of pH

The optimum pH was checked out for the samples at four different pH and it was found that 7.2 is the optimum for all samples.

CONCLUSION

From this study, it is clear that the leaves of *Coriandrum sativum*, *Nicotiana tobaccum*, *Murraya koenigii*, and *Moringa oleifera* can be used as a good source of protease enzyme. Apart from protease, the leaves of these plants can also be investigated for other industrially important enzymes. Very limited work has been done on enzyme production using these leaves which calls for a more detailed research in future. The other parameters for this enzyme can also be standardized for maximum production at a cheaper rate. There is a need to standardize all the other parameters including the cost effectiveness involved in the production of this enzyme.

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