



Research Article

ISSN : 0975-7384  
CODEN(USA) : JCPRC5

**Production and analysis of protease and amylase from *Aspergillus niger* using crab and prawn shell as substrate**

**L. Jeyanthi Rebecca<sup>\*</sup>, S. Sharmila, Merina Paul Das, T. V. Rishikesh and S. Anandanarasimhan**

*Department of Industrial Biotechnology, Bharath University, 173, Agharam Road, Selaiyur, Chennai-600 073.*

---

**ABSTRACT**

*About 1,234,332 tons of marine food is consumed on a weekly basis all around the world leading to the production of marine food processing waste like fish scales, crab and prawn shells, fins bones etc. In the present study crab and prawn shells were collected from a local fish market powdered and used as a substrate for *Aspergillus niger* and the activity of protease and amylase were analysed after three days. Two mutated strains of *A. niger*, were also used apart from the wild type *A. niger* strain.*

**Keywords:** protease, fish scales, enzyme activity

---

**INTRODUCTION**

Crustaceans, form a very large group of arthropods usually treated as a subphylum, which includes such familiar animals as crabs, prawns, crayfish, krill and barnacles. Like other arthropods, crustaceans have an exoskeleton, which they moult to grow. They are distinguished from other groups of arthropods, such as insects, myriapods and chelicerates, by the possession of biramous (two-parted) limbs, and by the nauplius form of the larvae. More than 10 million tons of crustaceans are produced by fishery or farming for human consumption, the majority of it being shrimps and prawns.

Industrial enzymes have seen a spectacular rise in their production in the last three decades. The growth of industrial enzyme market has expanded to nearly 85 enzymes, which are currently in commercial production. With the discovery of a variety of new and more active enzymes, the enzyme market has been forecasted to go upto US \$ 1.7-2.0 billion by 2006 [1].

Proteases, which account for about 60% of total enzyme market and among the most valuable commercial enzymes, are the single largest class of enzymes occupying a pivotal position due their wide application in the industrial processes [2,3]. Plants, animals and microbial sources are employed for protease production [4,5]. Microbes serve as the preferred source of proteases because of their rapid growth, the limited space required for their cultivation, and the ease with which they can be genetically manipulated to generate new enzymes with altered properties. Microbial extracellular alkaline proteases are important enzymes and are mainly used in detergents to facilitate the release of proteinaceous stains such as blood, milk, egg and meat. They account for approximately 40 % of the total worldwide enzyme sale.

Though a good number of bacterial alkaline proteases such as subtilisin Carlsberg, subtilisin BPN and Savinase are commercially available, having their major application as detergents enzymes but alkaline proteases of fungal origin offer an advantage over bacterial proteases because (i) the mycelium can be easily removed from the final product by simple filtration, (ii) ability of the fungus to grow on cheaper substrate, (iii) easy immobilization of mycelium for

repeated use, (iv) broad range of pH (4-11) and substrate specificity and hence low cost of production. As only few reports are available on the use of fungal proteases in detergent industry, there is a growing need to exploit fungal proteases for commercial exploitation in detergent industry.

For industrial use enzyme must be produced at low cost and should be reused reproduce result with consistent efficiency. To achieve this many techniques for immobilization of enzymes on different types of supports have been developed [6]. The immobilization of proteases on solid supports has been widely used in many investigations [7]. When a protease is immobilized, enzyme autolysis is minimized. For industrial applications, immobilization of the enzyme in gel or solid supports may offer several advantages such as repeated use of the enzyme, ease of product separation and improvement of enzyme stability [7]. The main objective of this study was to determine the protease and amylase activity from *A. niger* grown on powdered prawn and crab shells and to compare the enzyme activity of the wild-type with the two mutated strains of *A. niger*.

## EXPERIMENTAL SECTION

### Collection of sample

The samples were collected from a local fish market that regularly throws about 20Kg of crab and prawn shells per week as waste. Fig 1 and Fig 2 shows the picture of the samples used in this study.



Fig 1 crab shell



Fig 2 Prawn shell

They were washed with few drops of Tween 20 and then sterilized by continuously passing hot steam over it and drying it on exposure to sunlight for a day. It was then powdered and autoclaved.

### Test organism

Nutrient agar was prepared and autoclaved and then it was plated on sterilized petriplates and it was inoculated with a fresh pure culture of *Aspergillus niger*. The medium was kept for incubation for 3 days at 37°C and the plates were stored in the refrigerator. Two of the three plates were exposed to UV-rays for 5 and 10 sec respectively to get mutated strains.

### Inoculation of medium

The fungus were inoculated in test tubes containing 1gram each of the powdered shells and 1ml of 10% TCA buffer was added for maintaining the moisture content for the growth of the microbe. The test tubes were kept for incubation inside a incubator for 3days at 37°C. After incubation 2ml of TCA buffer was added to elute the enzyme.

### Enzyme Extraction

The sample was centrifuged at 10,000 rpm for 15min and the supernatant was collected. The collected supernatant was labeled and stored separately. For protease activity 0.5ml of the collected enzyme was mixed with 0.5ml of casein substrate and centrifuged at 3000 rpm for 10 min. For amylase activity 0.5ml of the collected enzyme was mixed with 0.5ml of starch and incubated for an hour. 1ml of TCA was added and centrifuged at 3000 rpm for 10min. The absorbance was checked spectrophotometrically at 660 and 620 nm respectively.

## RESULTS AND DISCUSSION

In this study the enzyme activity of samples had been analysed and the analysed data are tabulated in Tables 1 and 2 for protease and amylase respectively. The optical density of the enzyme samples were measured at 660nm and 620nm respectively in a UV-vis spectrophotometer.

**Table1: Protease activity**

Samples	Wildtype	UV treated 5sec	UV treated 10 sec
Prawn	0.923	0.635	0.597
Crab	0.705	0.565	0.546

**Table 2 : Amylase activity**

Sample	Wildtype	UV treated 5sec	UV treated 10sec
Prawn	0.261	0.458	0.232
crab	1.5672	0.811	0.741

Table 1 represents protease activity and Table 2 represents amylase activity of both Crab and Prawn shells. The wild type strain showed more activity when compared to the mutated ones.

### CONCLUSION

In this study we had found that the crab and prawn shell specimens had shown variation in the protease and amylase activity by wild type and UV mutated samples of *A.niger*. The UV mutated strains had not shown any significant result in the activities of the respected enzymes. The present study has only shown us some light on the possibility of enzyme production using crab and prawn shells as substrate. More work has to be done on the strain development.

### REFERENCES

- [1] T Godfrey; JR Reichelt, *Industrial Enzymology* **1983**, Nature press. New York, 1-7.
- [2] ED Brown; RY Yada, *Agric. Biol. Chem.*, **1991**, 55, 1639-1641.
- [3] J Escobar; SM Barnett, (**1993**). *Enzyme Microbial Technology*, 1993, 15, 1009-1013.
- [4] S Sharmila; L Jeyanthi Rebecca; V Dhanalakshmi; G Susithra and Md Saduzzaman, *International Journal of Applied Biotechnology and Biochemistry*, **2012**, Vol 2 (1): 81-85.
- [5] S Sharmila, L Jeyanthi Rebecca, Merina Paul Das, and Md Saduzzaman. *Journal of Chemical and Pharmaceutical Research*, **2012**, Vol 4 (8): 3808-3812.
- [6] VG Janolino; HE Swaisgood, *Biotechnol Bioeng.*, **1982**, 624:1069-80.
- [7] HE Swaisgood; GL Catignani, *Meth Enzymol.*, **1993**, 135: 596-604.