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Some Properties of Protease of the Fungal Strain *Aspergillus flavus*

MD. TOWHID HOSSAIN, FLORA DAS, L.W. MARZAN, MD. SHAFIQUR RAHMAN AND M.N. ANWAR[1]
Department of Microbiology, University of Chittagong, Chittagong–4331, Bangladesh
[1]Corresponding author's e-mail: anwarmn54@yahoo.com

ABSTRACT

The enzyme activity of a locally isolated fungal strain *Aspergillus flavus* was found maximum at pH 8.0 and 45^0C using casein as substrate and the enzyme was stabled at wide range of pH. The enzyme activity was inhibited by PMSF, which indicates that it belongs to the serine protease family. The molecular weight was found 46 kDa.

Key Words: *Aspergillus flavus*, Proteases; Fungal strain

INTRODUCTION

Microbial protease plays a significant role in diverse industries. Although a several proteolytic fungi and bacteria are known, few provide high activities with commercial success. Bulk production of enzymes has been reported in *Bacillus* sp. (Priest 1977); *Aspergillus niger*; (Bosmann 1973); *Aspergillus nidulans* (Stevens and Stevens 1980) and *Phycomyces blakesleeanus* (Fischer & Thomson 1979).

In some species only one type of protease has been reported, but in most species at least two or some times more types of proteases are produced, although not always under the same culture conditions. High temperature resistant thermophilic proteases, with their high specific activities (at high temperature) and their superior chemical and physical stability like characteristics, would seem to be good candidate for current and future biotechnological applications (Trehan, 1997).

Our fungal strain *Aspergillus flavus* produced high level of extracellular alkaline protease under batch culture conditions. Here, we report some properties of protease of this strain.

MATERIALS AND METHODS

Microorganism. The strain *A. flavus* (AP_2) used in this study was obtained from laboratory of Microbiology, University of Chittagong, which was previously isolated from pulse sample (Flora, 2004)

Measurement of enzyme activity. Protease activity was assayed using the modified method of Hayashi *et al.* (1967), as followed by Meyers and Ahearn (1977), using casein as substrate. The reaction mixture, (containing 3 mL of 1% (w/v) casein in 3 mL 0.1M citrate phosphate buffer, pH 7.0, and 3 mL of suitably diluted enzyme) was incubated at 40^0C±1 for 1 hour. The reaction was stopped by the addition of 5 mL 20% (w/v) TCA and the absorbance of the solution was measured at 650 nm in a spectrophotometer (Spectonic-21). The amount of amino acids released was calculated from a standard curve plotted against a range of known concentrations of tyrosine. One unit of enzyme was defined as the amount of enzyme that released 1µg of tyrosine mL^{-1} of crude enzyme h^{-1}.

Inhibition of protease activity. Inhibition of protease was determined using Phenylmethylsulfonyl fluoride (PMSF), EDTA, antipain, and 1,2- epoxy –3- (*P*- nitrophenoxy) propane (Brock *et al*., 1982).

Enzyme purification and determination of molecular mass. The crude enzyme from *A. flavus* was purified using ammonium sulfate at 60% saturation. The precipitates were collected by centrifugation at 12,000 rpm for 20 minutes at 4^0C and re-suspended in 0.1 M sodium phosphate buffer, pH 7.0. The molecular mass of the purified enzyme of *A. flavus* was determined using 12% SDS –PAGE gels (Laemmli 1970) at constant voltage for 3 h using standard protein markers of bovine serum albumin (66 kDa), casein (23.5 kDa) and egg albumin (45 kDa). The protein bands were visualized with coomassie blue R 250 stain for 1 h and destained with SDS PAGE destaining solution (10% acetic acid and 40% methanol).

RESULTS AND DISCUSSION

Factors affecting protease activity. Different factors that are regulates on maximum activity of protease of *A. flavus* were investigated and described below.

Enzyme-substrate reaction time. The optimum enzyme substrate reaction time was determined by incubating the reaction mixture at different incubation times (i. e. 10, 20, 30, 40, 50, 60, 70, 80, and 90 min.). The crude enzyme of *A. flavus* gave highest protease activity when incubated at 45^0C±1 for 60 minutes (Data not shown).

Substrate concentration. The activities of the crude protease from *A. flavus* at various substrate concentrations (i.e. 0.5 to 5.0% of casein solution) were observed and maximum activity was obtained with 3% casein as substrate (Data not shown).

Fig. 1. Effect of different substrate on activity of protease of *A. flavus*

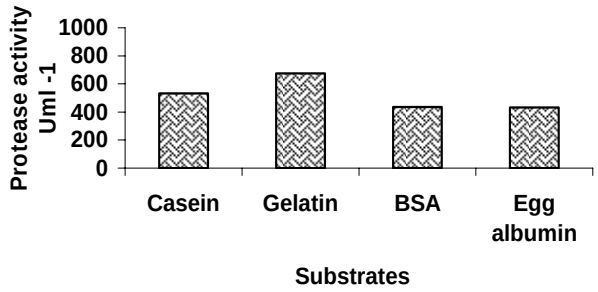


Fig. 2. Effect of pH and temperature on activity of protease of *A. flavus*

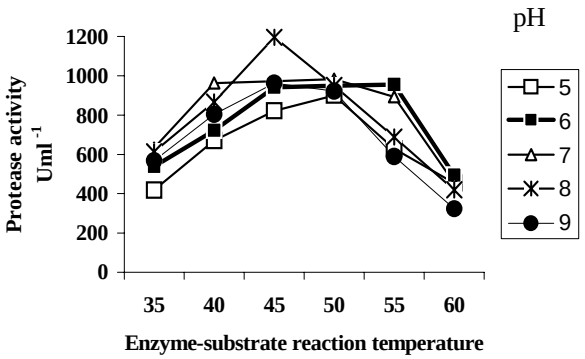
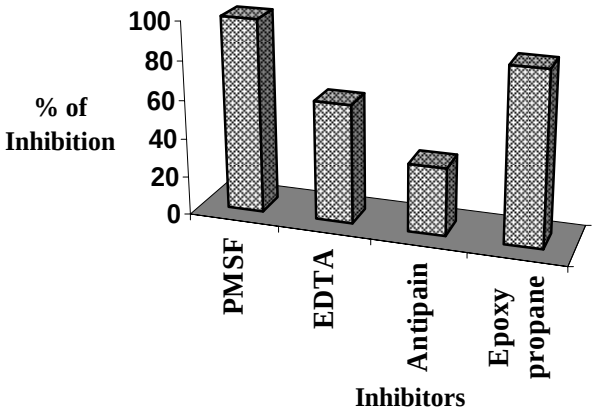


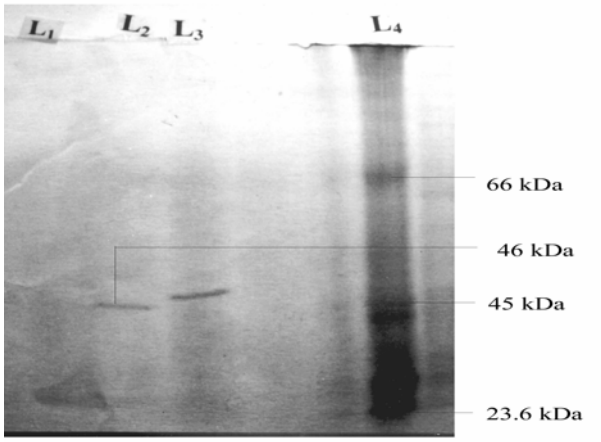
Fig. 3. Effect of inhibitors on activity of protease of *A. flavus*



Substrate specificity. The efficiency of enzyme activity on casein, gelatin, BSA and egg albumin was determined; highest values for activity were obtained with gelatin (Fig. 1)

Enzyme-substrate reaction pH and temperature. The effect of pH and temperature on protease activity was determined by incubating the reaction mixture at pH values ranging from 5.0 to 9.0 using 0.1M-citrate phosphate buffer at 35, 40, 45, 50, 55 and 60°C±1. Maximum protease activity of the crude enzyme extract of *A. flavus* was

Fig. 4. SDS-PAGE of purified enzyme of *A. flavus*. Lane-2 shows the purified enzyme, 46 kDa and lane-4 shows the molecular markers-bovine serum albumin, 66 kDa ; egg albumin, 45 kDa; casein, 23.6 kDa.



recorded at 45^0C±1 and pH 8.0 with 3% casein as substrate (Fig. 2). Most alkaline proteases have been reported to have optimum activity in the range pH 8-9 (Gusek & Kinsella, 1987). Protease activity at 45^0C was also reported by Shumi *et al.* (2003) while working with the protease of *Fusarium tumidum.*

Inhibition of protease activity. The crude enzyme of *A. flavus* showed 100% inhibition in the presence of PMSF indicating that the enzyme is an alkaline serine proteinase (Fig. 3). Work on serine proteinase has been reported by many researchers (Morihara, 1974; North, 1982).

Enzyme purification and molecular mass. Using SDS-PAGE, the semi purified enzyme *A. flavus* showed a single band (Fig. 4), to confirmation it as a enzyme protein band, the protease activity of purified enzyme (eluted & unstained) was also observed and the apparent molecular mass of the purified protease was 46 kDa. In this respect, it seems to be protease of *A. flavus* closely related to the protease enzyme of *Pseudomonas* strain P-24 reported by Hoshino *et al.* (1997).

We have characterized an alkaline serine proteinase from a locally isolated fungus, *A. flavus*. Its desirable characters such as broad substrate specificity, stability at high alkaline pH, stability at high temperature are significant characteristics of any enzyme for industrial application.

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