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IMPACT OF PROTEASE INHIBITORS ON EXTRACELLULAR CRUDE PROTEASE ACTIVITY OBTAINED FROM DIFFERENT STRAINS OF *ASPERGILLUS*

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ABSTRACT

Proteases are hydrolytic enzymes which catalyze the cleavage of peptide bond in other proteins. They have a wide range of applications particularly in food, detergent, weave, leather, pharmaceutical and chemical industries.

In the present investigation protease inhibition studies were done. It gives an insight into nature of an enzyme produced by different strains of *Aspergillus*. Of the inhibitors tested PMSF was able to inhibit the crude protease activity obtained from all the *Aspergillus* strains and B-ME enhances the crude protease activity indicating that they all are thiol dependent serine proteases.

Keywords: Protease, *Aspergillus,* PMSF, Serine protease

INTRODUCTION

Proteases are degradative enzymes that hydrolyze peptide bonds in other proteins. Proteases represent the class of enzymes which occupy a pivotal position with respect to their physiological roles as well as their commercial applications.

They perform both degradative and synthetic functions **[1]**. Microbial proteases are more useful than other sources due to easy methods of production as well as their vulnerability to genetic manipulation **[2]**. They are well used in detergent, food, pharmaceutical, chemical, leather and silk industries as well as in environment **[3]**.

Proteases are classified on the basis of three major criteria, type of reaction catalyzed, chemical nature of the catalytic site and evolutionary relationship with reference structure. Proteases are grossly divided into two major groups , i.e. Exopeptidases cleave a terminal amino acid residue at the end of polypeptide; endopeptidases cleave internal peptide bonds. Hooper **[4]** provides a useful introduction to the general properties of proteases. Proteases can be classified based on the chemical groups that function in catalysis. In serine proteases the hydroxyl group in the side chain of a serine residue in the active site acts as a nucleophile in the reaction that hydrolyzes a peptide bond, whereas in cysteine proteases the sulfhydryl group of a cysteine side chain performs this function. In aspartic acid proteases and metalloproteases, a water molecule in the active site (positioned by interacting with an aspartyl group or a metal ion, respectively) functions as the nucleophile that attacks the peptide bond.

In the present investigation impact of various protease inhibitors on the protease activity obtained from *Aspergillus* strains was studied which gives an insight into the nature of an enzyme.

MATERIAL AND METHODS-

Fungal strain- *Aspergillus* strains were isolated from soil on Potato dextrose agar medium and screened for protease production on Yeast Extract Agar Medium.

Enzyme Production- The enzyme was produced in conical flasks containing 100 ml of protease production medium (Yeast Extract broth containing 1% casein), incubated at 28^{o} C for 7 days in an orbital shaker (BOD incubator, MAC, India). Cell free cultural filtrate was used as a crude enzyme solution.

Protease assay- The crude protease activity was determined by the method of Anson **[5]** and Folin Ciocalteue **[6]**. Protease activity was determined in a reaction mixture of 1 ml enzyme, 5 ml (0.65mM) casein solution (pH 7.5) and incubated at 37° C for 10 minutes. The proteins were precipated by adding 5 ml of TCA and free amino acids released by protease from casein hydrolysis were estimated. One protease unit is defined as the amount of enzyme that release 1.0 micromol (181 microgram) of tyrosine per minute at pH 7.5 at 37° C (Colour by Folin Ciocalteu Reagent).

Effect of protease inhibitors and chelators on Enzyme activity- The effect of various protease inhibitors (5mM) such as serine inhibitors [Phenylmethyl suphonyl fluoride (PMSF)]; Cysteine inhibitors [p-Chloromercuric benzoate (pCMB)]; and [B-Mercaptoethanol (B-ME)}, Iodoacetate and a chelator of divalent cations {ethylene diamine tetraacetic acid (EDTA) were determined by preincubation with the enzyme solution for 30 mins at 37 °C before the addition of substrate. The relative protease activity was assessed quantitatively.

RESULTS AND DISCUSSION –

Inhibition studies primarily give an insight into nature of an enzyme, its cofactor requirements and the nature of the active centre **[7]**. The effect of different inhibitors on the enzyme activity of crude protease was studied quantitatively **(Table 1)**. Of the inhibitor tested at (5mM concentration), PMSF was able to inhibit the crude protease completely from *Aspergillus* strains no. 3, 10, 11, 13 and 14. In this regard, PMSF sulphonates the essential serine residue in the active site of the protease and has been reported to result in complete loss of enzyme activity **[8]**. Our findings were similar to those of Hossain *et al*., **[9]** for protease produced by *Aspergillus flavus* and Sharma *et al*., **[10]** for AWT20, where the protease was inhibited by PMSF. This indicates it is a serine protease. In the case of other inhibitors, p-CMB inhibit protease produced by all the strains of *Aspergillus* strains which indicates that there is also role of cysteine in enzyme activity. On the other hand Iodoacetate has no inhibitory effect or very slight increase in crude protease activity as shown in table-1. B. Mercaptoethanol enhances the protease activity produced by all the strains of *Aspergillus* indicating that they are thiol dependent serine proteases **[11]**. EDTA inhibits the enzyme activity produced by isolate no S-11, S-13 and S-14 whereas there is no stimulatory or inhibitory effect on protease activity from isolate S-3 and S-10. para-Cholоromercuribenzoate has no effect either inhibitory or stimulatory on protease activity from all the strains of *Aspergillus.*

Table 1: Residual relative enzyme activity (in percent)-Control- 100%

Inhibitors / Strains	p-CMB	EDTA	PMSF	B-ME	IAA
S-3	98	102	91	151	102
S-10	101	100	89	131	99
S-11	100	78	92	131	103
S-13	97	90	90	110	101
S-14	99	89	65	114	102

CONCLUSION

From the above result it can be concluded that proteases produced by all the strains of *Aspergillus* are mixed type thiol dependent serine proteases.

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