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EXPRESSION, PRODUCTION AND PURIFICATION OF PROTEINASES FROM *ASPERGILLUS* SP.

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ABSTRACT
Screening and expression of protease producing 18 strains of *Aspergillus* sp. were obtained from the various places in Chennai, Vellore, Tamilnadu, India. The isolates were positive on tyrosin caesin nitrate agar (1%) and thus are selected as protease producing strain. The microbial growth is revealed by the mycelial dry weight determination. Maximum growth is observed in the case of *Aspergillus flavus* (0.64 mg). Equally, the maximum growth is observed in *Aspergillus sojae* (0.63 mg). Total protein was determined using BSA as standard. Proteinase activity was estimated using casein as the substrate. Finally the enzyme protease was purified by column chromatography. The protein was characterized using SDS-PAGE. Maximum protein content is observed in the case of *Aspergillus tamarii* (0.514mg) and *Aspergillus awamori* (0.461 mg) Maximum proteinase content is observed in *Aspergillus nidulance* (0.866 mg). This results showed that microbes under study is a good producer of extra cellular protease, which can be beneficial for industries.

KEYWORDS: Mycelial dry weight, *Aspergillus*, chromatography, protein content, Proteinase.

INTRODUCTION

Enzymes are delicate protein molecules necessary for life. Proteinases are one of the most important industrial enzymes found in wide variety of microorganisms. They are molecules of relatively small size, spherical structures that catalyze the peptide bond cleavage in proteins (Polgar, 1989). These enzymes are important in a number of biological processes viz regulation of metabolism (Rao et al., 1998). Gene expression, pathogenicity and biological application. It also find application in leather industry, food industry, pharmaceutical and bioremediation process (Anwar and Saleemuddin, 1998; Gupta et al., 2002). The largest application of proteases is in laundry detergents, where they help removing protein based stains from clothing (Banerjee et al., 1999), waste processing industries (Pastor et al. 2001). Panicker et al, (2009) studied the purification and characterization of serine proteases from mature coconut endosperm, while characterization of asparaginyl proteinase were investigated by Oliver et al (2006). The present attempt was aimed to compare the changes in the production and activity of these useful enzymes proteinases from different species of *Aspergillus.*

MATERIALS AND METHODS

Collection and isolation of sample

About 18 strains of *Aspergillus* were obtained from the culture collection centre of Botany Department of CAH college of Melvisharam, CAS Botany, University of Madras, Dept of Biotechnology CLRI and PG. Extension Centre, Vellore and other Microbiology Laboratories nearby. A few strains were isolated from the air microflora of Kancheepuram and cultured in Tyrosin caesein nitrate agar (Annadurai et al., 1989).

Mycelial dry weight determination

Mycelial mats formed from microbial cultures were filtered with coarse grade glass filters with water and transferred to a previously weighed filter paper and dried at 70° C overnight and cooled at room temperature in a desicator and weighed to a constant weight correct to the mg values given in the tables are mean of triplicate values Annadurai et al., (1998, 1999, 2000).

Preparation of Crude extract

The microorganisms along with the culture medium were triturated in a mortar with acid-washed sand. This was centrifuged and the supernatant was collected. The residue was triturated again with distilled water, centrifuged and the supernatant was mixed with the previous extract. The total liquid collected was made up to a known volume and analyzed for proteolytic activity.

Estimation of Protein

Total protein was determined by the method of Lowry et al., (1951) using BSA as the standard. Sample containing 50-100µg protein were mixed with 5ml of alkaline copper solution (freshly prepared with 50 ml of 1% Na_2CO_3 in 0.1 N NaOH and 1 ml of 0.5% $CuSO_4$. $5H_2O$ in 1% potasium tartrate) and kept for 10 mins at room temperature. It was then mixed with 1 ml of Folin-Ciocalteau's phenol reagent and allowed to stand for 30 mins and the blue color developed was read at 640 nm.

Determination of Proteinase enzyme

Proteinase activity was estimated according to the method of Kurnitz (1947) using case in as the substrate. In this method, a protein substrate is subjected to enzymatic hydrolysis. The tyrosine liberated by the hydrolysis is quantitatively estimated by measuring the absorbance at 275 nm. The amount of tyrosine liberated is directly proportional to the enzyme activity.

1ml of casein solution (1% w/v in 0.1 m Tris-HCl buffer pH 9.0) was mixed with 0.5 ml of suitably diluted enzyme solution and incubated at 45 C for 10 min. 50% TCA was then added to arrest the enzyme action. The mixture was warmed and filtered through whatman no.1 filterpaper. The filterate was read at 275 nm. A control was run in an identical manner except that the enzyme was added after the addition of TCA

Enzyme Purification

Ammonium Sulphate Precipitation

The organism was grown for 48 hours as described previously. The cells were separated by centrifugation (10 000 rpm, 15 minutes), and the supernatant was fractionated by precipitation with ammonium sulfate between 50% and 70% of saturation. All subsequent steps were carried out at 4°C. The protein was resuspended in 0.1M Tris-HCl buffer, pH 7.8, and dialyzed against the same buffer. Further purification of dialyzed protein was done by column of Sephadex G-200, **SDS-PAGE**

The elution profile of protease by Sephadex G-200 column Chromotography showed, the dialyzed ammonium sulphate precipitates loaded on to a DEAE Sephadex G 200 column. Proteins eluted are collected and tested for specific protease activity. The absorption peaks at 280 nm were observed. The column purification shows greatest activity of enzyme. Eluted samples from Sephadex column where run on a 12% SDS page stained with silver. The band obtained with the molecular weight of 60,000 Da as protease activity band.

RESULTS

Fig.1 and Tab.1 shows the mycelial dryweight, protein content and proteinase activity of different species of *Aspergillus* in culture filtrate. The growth is revealed by the mycelial dryweight determination. Among 18 species of *Aspergillus* tested, maximum amount of dryweight is observed in *Aspergillus flavus.* Equally good amount of growth is seen in *Aspergillus sojae.* The protein content is maximum in *Aspergillus tamari.* Good amount of protein content is also observed in *Aspergillus awarmori.* The extracellular proteinase activity is maximum in *Aspergillus nidulance.* Good amount is also noted in *Aspergillus oryzae.*

Fig.1. MYCELIAL DRYWEIGHT, PROTEIN CONTENT AND PROTEINASE ACTIVITY OF *ASPERGILLUS* SPECIES

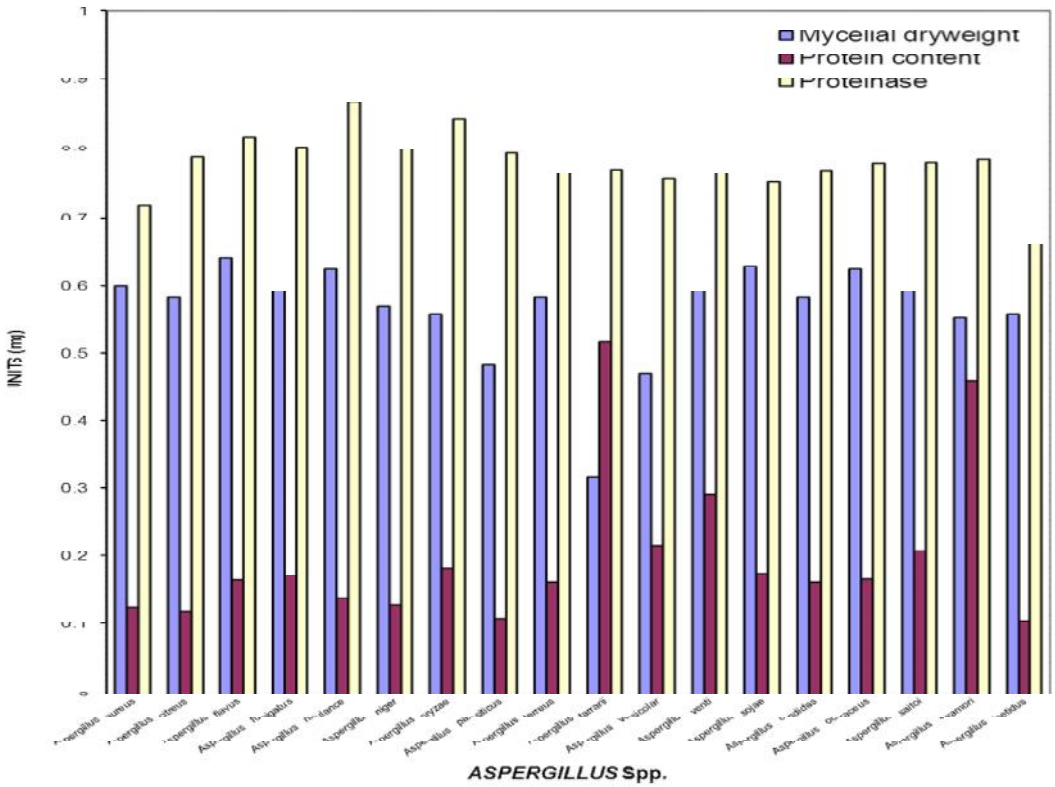

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TABLE 1 Mycelial Dry Weight, Protein content and Proteinase activity of *Aspergillus* species

Name of the Genus	Mycelial dry weight	Protein content	Proteinase activity
Aspergillus aureus	0.6	0.126	0.716
Aspergillus citreus	0.58	0.121	0.787
Aspergillus lavus	0.64	0.165	0.817
Aspergillus fumigatus	0.59	0.176	0.804
Aspergillus nidulance	0.62	0.143	0.866
Aspergillus niger	0.57	0.129	0.798
Aspergillus oryzae	0.56	0.185	0.845
Aspergillus parasiticus	0.48	0.113	0.792
Aspergillus terreus	0.58	0.162	0.765
Aspergillus tarrarii	0.32	0.514	0.772
Aspergillus versicolar	0.47	0.219	0.755
Aspergillus venti	0.59	0.293	0.764
Aspergillus sojae	0.63	0.178	0.751
Aspergillus candidas	0.58	0.162	0.771
Aspergillus ochraceus	0.62	0.166	0.779
Aspergillus saitoi	0.59	0.213	0.78
Aspergillus awamori	0.55	0.461	0.784
Aspergillus foetidus	0.56	0.11	0.658

Finally the enzyme proteinase was purified by column chromatography Fig. 2 shows the elution profile of protease by G 200 column chromatography. Proteins eluted all are collected and tested for protein activity. The column purification shows greatest activity of enzyme.

FIGURE 2. Elution profile of Protease by Sephadex G –200 Column

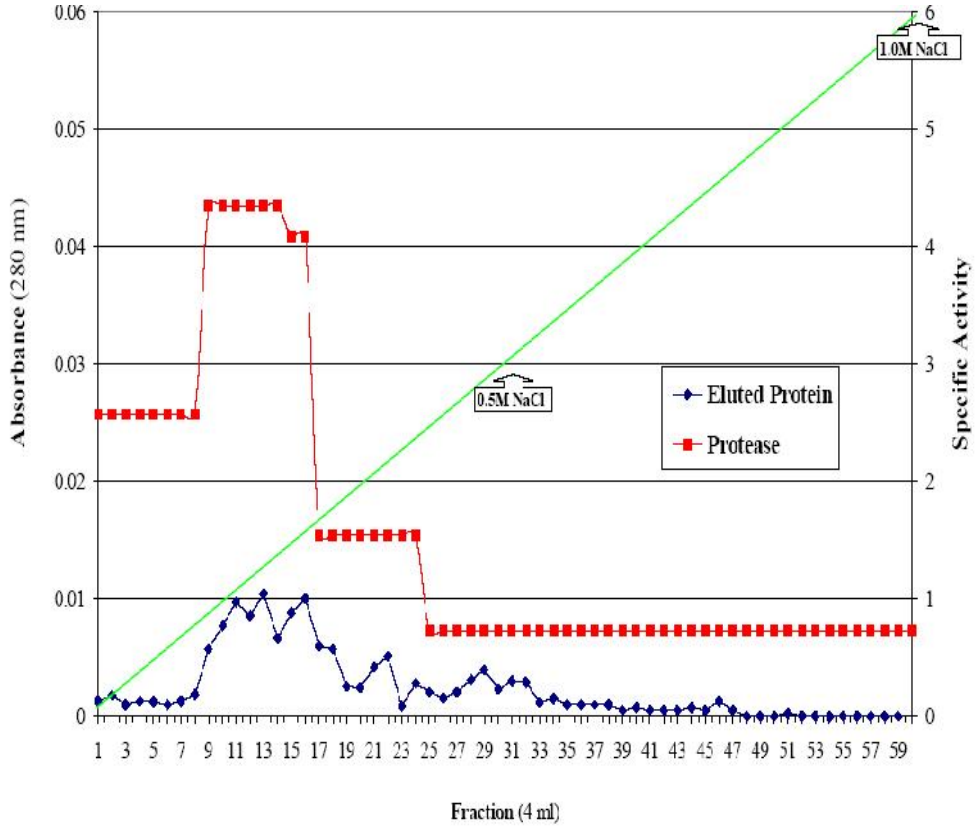
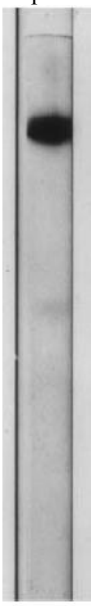


FIGURE 3. indicates the electrophoresis of proteinase. Eluted samples from sephadex column were run on 12% SDS polyacrylamide gel stained with silver. The band obtained with the molecular weight of 60,000 Da as the protease activity band.

FIGURE 3. Electrophoresis of Proteinase



DISCUSSION

In recent times, *Aspergillus* proteinases are receiving much attention, because of their increasing applications in chemical, detergent and leather industries. The new field of enzyme engineering has in a short period of time made striking contribution to industry, medicine, agriculture and in pollution control. proteinases are obtained from selected strains of *Aspergillus* molds and bacteria Among the strains, *Aspergillus nidulance, Aspergillus flavus, Aspergillus oryzae, Aspergillus fumigates* and *Aspergillus awamori* are producing proteinases. Among the bacterial strains, *Bacillus* is the most useful source for the production of proteinases. Proteinase is particularly suitable for industrial use, as it can be produced economically on a large scale, by controlled fermentation. While concentrating the large scale of production of proteinase enzyme only 18 species of *Aspergillus* have taken for this investigation. Out of which only 5 to 6 *Aspergillus* sp. are turned to be very good sources for the proteinase enzyme production. These microorganisms through cultural methods have suggested for the large-scale production of proteinase enzyme in future.

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