

Extraction and purification of protease from *Aspergillus niger* isolation

Abstract

Growth *Aspergillus niger* on solid state fermentation media for produce protease. The best condition for protease production in the moisture is 1:30 and incubation at 40°C for 8day at PH 8. Inoculation with 2.2×10^4 cell/ml of fungal media containing, different nitrogen sources the peptone the best. Protease was precipitated from production media by 70% saturation of ammonium sulphat with 3.5 U/mg protein specific activities, the final purification in DEAE-cellulose 9.8 fold of purification and 0.188 yield.

Keywords: *Aspergillus niger*, protease, purification, enzyme

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Mais E Ahmed

Department of Biology, University of Baghdad, Iraq

Correspondence: Mais E Ahmed, Department of Biology, College of science, University of Baghdad, Iraq, Email mais.e.mahmood@gmail.com

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Introduction

Industrial enzymes have seen spectacular rise there production in the last 3 decades. The growth of industrial market has expanded to nearly 85 enzymes, which are correctly in commercial production with the discovery of variety of new and more active enzyme. Protease represent one of three largest group of industrial enzyme and account for about 60% of the total worldwide sell of enzymes.¹ Proteases are the single class of enzymes which occupy a pivotal position with respect to their applications in both physical and commercial fields.² Proteolytic enzymes catalyzed the cleavage of peptide bonds in their protease, proteolytic enzymes can be classified as acidic³ natural and alkaline protease with the regard to their PH working range. Natural and alkaline proteases hold great potential for application in the detergent and leather tanning industrial due to increasing trend in developing environment friendly technologist.⁴ Alkaline proteases have numerous applications in food industry.⁵ Silver recovery from x-ray film⁶ and several bioremediation processes. Plants, animals and microbial sources are employed for protease production. Microbes serve as the preferred source of proteases because of their rapid growth the limited space required for their cultivation, and the ease which they can be genetically manipulation to general new enzymes with prosperities⁷ microbial extra cellular protease are important enzyme and mainly used in detergent to facilitate the release of protein strain such as blood, milk, egg, meat and food and leather industrial and is an important tool in studying the structure of protein and peptide.^{8,9} The price of commercially available enzyme which are produced mostly by submerged fermentation usually too high for biotechnology applications. An alternative technique for enzyme production is solid state fermentation.¹⁰ Solid state fermentation has some advantage compared with liquid state culture higher product yield, better product quality, cheaper, product recovery and cheaper technology.¹⁰

Materials and methods

Microorganism and culture conditions

Aspergillus niger was obtained from biology department/ college of science / Baghdad university, the organism maintained on potato-dextrose agar slant at 4°C.

Preparation of culture filtrate

Wheat bran under the conditions of solid state fermentation Erlenmeyer corucal flask of 250 ml capacity were used containing 20 ml of liquid media without organic source and 10 gm of dry wheat bran medium and sterilized at 21°C–15 minutes inoculation spore suspension of organism preparation sterile distilled water and adjusted to (1×10^6) spores/ml. incubation was carried out 26°C under static conditions after 7 days the contain of the flask were filtered through Whatman No. 3 filter paper disc. Culture filtrate was centrifuged at 5000 g for 10 mins/4°C and supernatant was used as the crude enzyme.

Proteins assay

Protease activity in the culture supernatant was determined by using in the method originally described by Murachi¹¹ and modified by Senior¹² as following:

Casein (0.8 ml, 0.5% and PH=8) was pre incubated in water bath at 37°C for 10 minutes. Unit of enzyme activity was defined as the amount of enzyme, which give 0.01 increase at absorbent $Ab=280$ nm/min under the assay condition. The protease activity was determinate by using this equation:

$$\text{Protease activity U/ml} = \text{Ab. at } 280 \text{ nm} / 0.001 \times 20 \text{ min.} \times 0.2 \text{ ml.}$$

One unit of protease activity was define as that amount of enzyme which librates 1 mg equivalent of peptide fragment under the assay condition using bovine serum albumin as standard specific activity is expressed unit per mg protein.

Optimization of the enzyme

PH: The natural protease production was determined by using different PH 5,6,7,8.

Incubation period: The natural protease production was determined by using different incubation period 7, 8, 9 and 10 days.

Temperature: The influence of temperature of production of natural protease was studied by incubating media at different temperature 25, 30, 35 and 40°C.

Nitrogen source: Nitrogen source of natural protease production was determined by using different sources (peptone, tryptone, casein and yeast extract).

Inoculum size: The influence of inoculation represent (1×10^6) of the production of natural protease was studied by inoculation media at the different volumes 1×10^6 , 2×10^6 , 3×10^6 and 4×10^6 spores/ml.

Purification of protease

Purification steps inoculated ammonium sulphate precipitate optioned 60-90% saturation was developed in small amount of 0.02 M sodium sulphate puffer PH 6.8 and was dialyzed 3 times over night against the same buffer. Any sediment formed were removed by centrifugation and the supernatant was loaded on DEAE-cellulose Colum 2×30 cm previously equilibrated with the 0.02 M sodium phosphate buffer, PH 7 column elution was performed by the same buffer with increase in morality form 0.02 to 0.5 M by inoculation of NaCl.

Protease activity was assayed at PH 6.8 and the peaks obtained were intern tested for optimum PH. Fraction showing maximum activity in the respective peak area were boiled.

Results and discussion

Influence of PH

The maximum protease production 3.4 U/ml found at PH 7 (Figure 1). The result clearly indicated neutrophilic of the fungus. The medium protease production by *A. niger* was observed in range PH 7-9. Growth and protein production cased of PH 10 optimum PH 7 has been reported for natural protease of *A. niger*.¹³

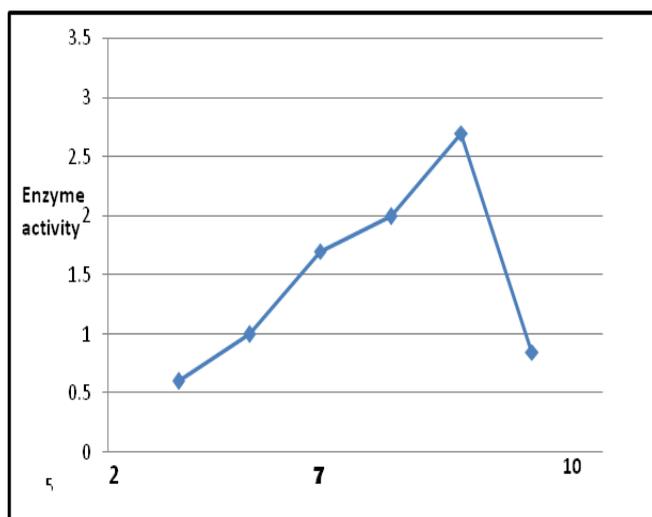


Figure 1 Effect of PH on the production of protease by *A. niger*.

Influence of incubation period

The maximum activity of enzyme was observed after 8 days of fermentation process (Figure 2) when the fungus grows passed the lag. Phase and the enzyme production start¹⁴ stated the *A. flavus* optimally produced protease after 10 days of incubation.

Influence of temperature

The protease production at different temperatures range were examined after 8 day keeping the other fermentation constant protease production increase at temperature 40°C maximum production of protease 4.2 U/ml obtain at 40°C (Figure 3) also found *Bhanerochaete chrusosporium* optimally at 25°C .¹⁵ It was revealed that temperature does not only affect growth rate of organism but also exhibited marked

influence on the level of protease production.

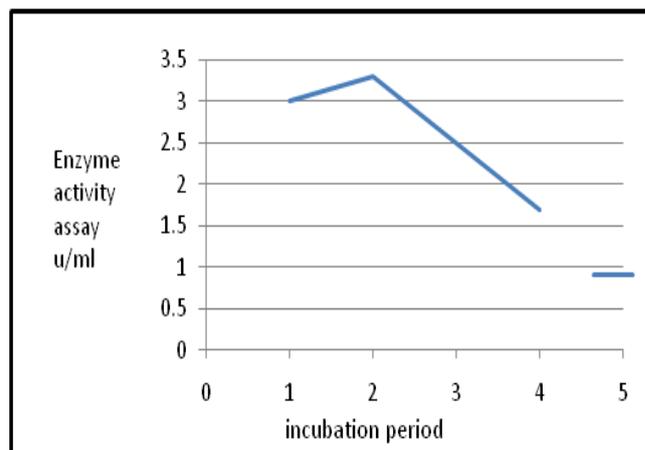


Figure 2 Effect of incubation period on the production of protease by *A. niger*.

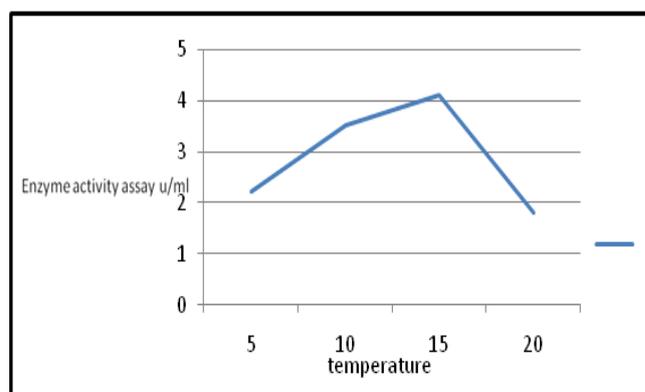


Figure 3 Effect of temperature on the production of protease by *A. niger*.

Influence nitrogen source

Among the various nitrogen source peptone and tryptone were found to be the most effective for protease product 5.4, 5.1 U/ml respectively (Figure 4). The mechanism that shows the formations of extracellular enzyme influenced by the visibility of precursors for protein synthesis. The effect of nitrogen source like peptone, casein, tryptone and yeast extract has reported that nitrogen source stimulated equal accumulation of protease in the culture medium of *A. terreus*.¹⁶

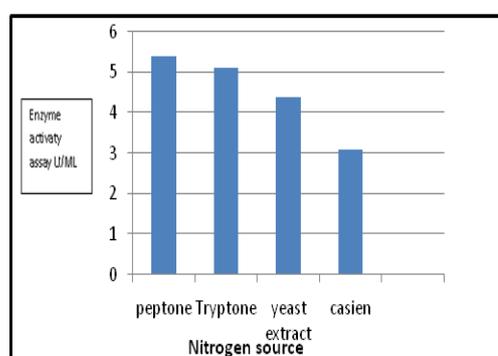


Figure 4 Effect of nitrogen source on the production of protease by *A. niger*.

Influence of inoculums size

The study of the effected of inoculums size, fungus was grown at various inoculums size (1×10^6 , 2×10^6 , 3×10^6 and 4×10^6 spores/ml.) using production medium containing 1% casein as the main substrate. The highest activity was observed for protease production 3.26 U/ml

in 1×10^6 spores/ml inoculums size of the spore suspension (Figure 5) in order to verify the enzyme activity, the spore concentration must be high enough to colonized the substrate particles,¹⁷ many studies however have indicated that there can be peak line in this activity over determent spore concentration.

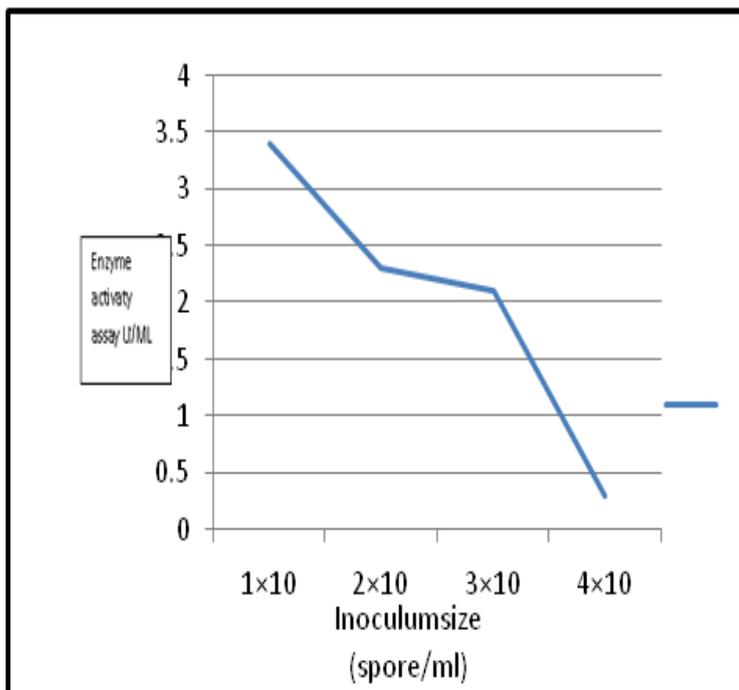


Figure 5 Effect of inoculums size on the production of protease by *A. niger*.

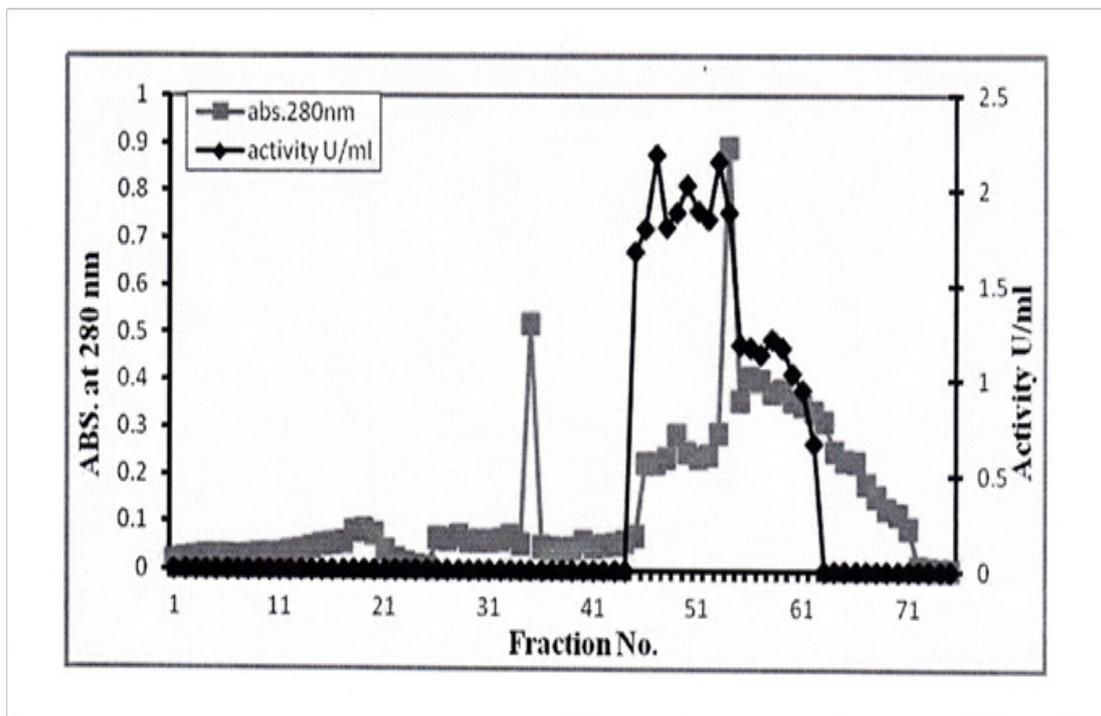


Figure 6 Elution profile of protease from DEAE-cellulose column chromatography.

Purifications steps

Protease produced by A.spp was recovered in Table 1. The first step was carried out by precipitation of protein from the cell free dialysis with ammonium sulphate at the saturation level of 60%. This resulted in 2.3 fold of purification of protease with yield of 9.78% of

the original activity chromatography on DEAE-cellulose using linear sodium chloride ingredient the wash step and until fraction no. 41 and then start elution step. The result showed that protease that protease was purified 8.94 fold to specific activity of 30.21 U/mg protein (Figure 6).

Table 1 Purification of protease from *A. niger* by solid state fermentation.

Sample	Volume(ml)	Activity(U/ml)	Protein (mg/ml)	Specific activity(u/mg)	Total activity (U/ml)	Total protein (mg)	Yield %	Fold
Crude extract	400	3.9	1.17	3.37	1580	468	100	1
Perception 60%	25	6.18	0.78	7.8	154.6	19.6	9.78	2.33
DEAE-cellulose	90	1.57	0.052	30.2	141.9	4.68	0.18	8.94

Conclusion

Purification enzyme protease by DEAE-cellulose from local isolation *Aspergillus niger* optimum condition produce at 2 day at PH 8 Inoculation with 2.2×10^4 cell/ml.

Acknowledgement

None.

Conflict of interest

None.

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