# Study on Cysteine Proteinase Produced by *Entamoeba Histolytica*

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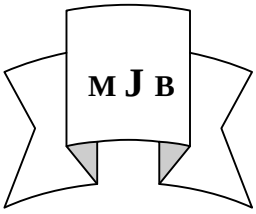


## Abstract

In this work , cysteine proteinase produced by *Entamoeba histolytica* trophozoite had been detected. The isolated enzyme had been immobilized by using calcium alginate, and compaired between free enzyme (mobile) and immobilized enzyme through differente range of pH and tempreture. We found that immobilized enzyme remains with its enzymatic activity through changing in pH level and temprature in compairion with free enzyme.

## الخلاصة

تم خلال هذا البحث التحري عن انزيم السستين بروتينيز المنتج من قبل الاطوار المتغذية للاميبا الحالة للنسيج. تم تقيد الانزيم المعزول باستخدام مادة الجينات الكالسيوم وقورن بين الانزيم الحر(الغير مقيد) والانزيم المقيد من خلال تعرضه الى ارقام هيدروجينية ودرجات حرارية مختلفة.وجد ان الانزيم المقيد يبقى محتفضا بفعاليته الانزيمية عند التغير في الارقام الهيدروجينية ودرجات الحرارة مقارنا مع الانزيم الحر.

## Introduction

Cysteine proteinases are a large group of enzyme which include lysosomal cathepsins. Physiologically cysteine proteinase have an important role in protein metabolism and turnover. In addition, processing and activation of many prohormones, proenzymes and peptides is conducted by cysteine proteinase[1].

Cysteine proteinase is considerd to be a key virulence factor which lysis the target cell [1]. Other virulence factors such as hyaluronidase, phospholipase and hemolysine are important in pathogenesis of amoebiasis [2]. Infection with *E.histolytic* can by controlled by inhibition the virulence factors especially cysteine proteinase [3] .

The action of cysteine proteinase can be regulated and inhibited by endogenous, natural inhibitors as well as compartmentalization and surrounding condition such as pH or chelating agents. If this well-balanced control system is disturbed and can lead to serious damage. Many pathogens have their own cysteine proteinase to invade hosts[3]. *E. histolytica* cysteine proteinase is a polypeptide chain which contains 447 amino acid and it is considered the important virulence factors produced by trophozoite of *E. histolytica*. It located in cytoplasmic vesicales and in the plasma membrane of the trophozoite [1]. Convincing evidence find that cysteine proteinases are essential for *E. histolytica* induced pathology.Treatment of amoeba with sublethal dose of a specific cysteine proteinase inhibitor or the addition of laminin which blocks the substrate –binding pocket of cysteine proteinase is greatly reduced its ability to produce liver abscesses in laboratory animals [4]. Moncada *et al*. (2003)[5] performed cysteine proteinase produced from trophozoite of *E. histolytica* have the ability to overcome the defence barrier of colonic epithelia by degrade mucin and invasive to epithelium layer.

## Materials and Methods

### 1- Detection of *E. histolytica* cysteine proteinase

Extracellular proteinase was detected by using $M_9$ medium supported with 0.5% casein. Crude enzyme was isolated and put in Petri dish by picking and patching methods, then incubated at 37Cº for 24-48 hours and 3 ml of trichloroacetic acid (T.C.A.) was added to each Petri dish for precipitation unlysis protein. Production of extracellular proteinase was indicated by observing a hallow around the wells. [6].

### 2- Immobilization of cysteine proteinase produces by *E. histolytica*

- **Concentration with ammonium sulphate**

Weights of ( 2.68, 5.82, 9.52 and 10.06) gm ammonium sulphate were added gradually to 20 ml of crude enzyme with mixing by magnetic stirrer under cold condition (ice bath) to obtain saturation percentage of 25%, 50%, 75% and 90% respectively. Crud enzyme was collected in sterile tube and centrifuged at 10000 rpm for 15 minute. The enzymatic activity was measured in supernatant and precipitate to determine the percentage of saturation. Then dialysis was done for 24 hours by using distilled water.

- **Immobilization of enzyme with Ca-alginate**

Entrapment methods with calcium alginate were used to immobilize cysteine proteinase isolated from *E. histolytica* trophozoite according to [7].

Two ml of crude enzyme was added to 10 ml of Na- alginate solution (4%) and mixed well by magnetic stirrer for 15 minute with cold condition, then put in syringe elongated with fine wire tube (3 mm diameter ). The mixture dropped on the surface of 1% calcium chloride ($CaCl_2$) to form beads with 3 mm diameter. These beads were washed with calcium chloride to remove unbounded enzyme, and then enzymatic activity measured in washing solution to determine the percentage of immobilization. Then enzymatic activity estimated according to [8].

## Results and Discussion

### 1- Cysteine proteinase detection

The results showed that the ability of trophozoite to produce cysteine proteinase extracellularly noticing the hyaline hallow around the well, then 3 ml of 5% Trichloroacetic acid (T.C.A.) was added to the culture media after the incubation period (figure 1). This results are consistent with the results of [1] and [9] who have demonstrated the ability of *E. histolytica* trophozoite to release extracellular cysteine proteinase *in vitro*. The secretion of cysteine proteinase extracellularly is one of the most important mechanisms for invasion the trophozoite of *E.histolytica* into the host tissue especially the intestinal layers lining the colonic epithelium and cleave the component of the extracellular matrix such as collagen, elastin and fibrinogen (Nozak and Nakada-Tuskui, 2005).

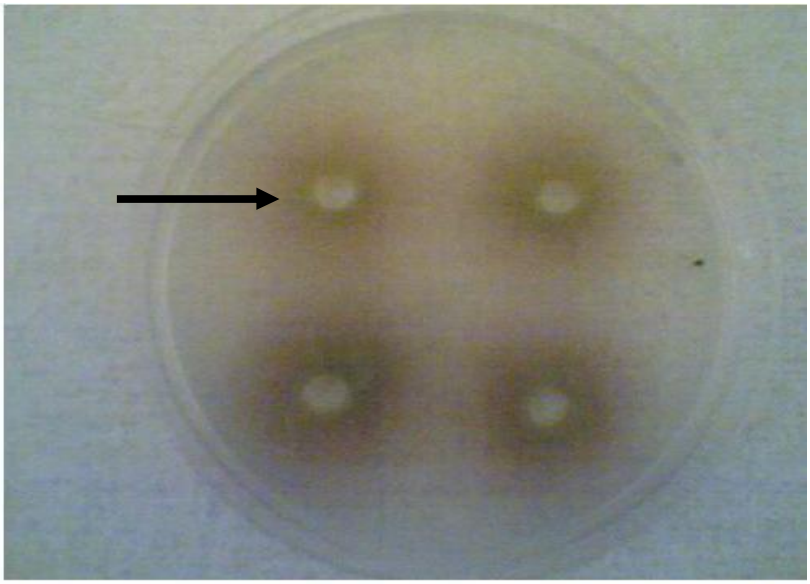

**Figure 1** Arrow should be explained ability of *E. histolytica* to produce cysteine proteinase

**2-cysteine proteinase immobilization**

We used Entrapment method in immobilized cysteine proteinase of *E.histolytica* by using calcium alginate (figure 2).

The results showed that Ca-alginate is very efficient in immobilization the enzyme since the percentage of immobilization reached up to 76% in which the enzyme maintain its activity. Beshay (2003) confirmed that entrapment in calcium alginate is the most suitable methods for cell immobilization and Ca- alginate is readily available and it is non toxic biological materials. The results of our study are in accordance with that results obtained by Al-Khafaji (2006) who used Ca- alginate in immobilized urase enzyme and the percentage of immobilization was 80%.

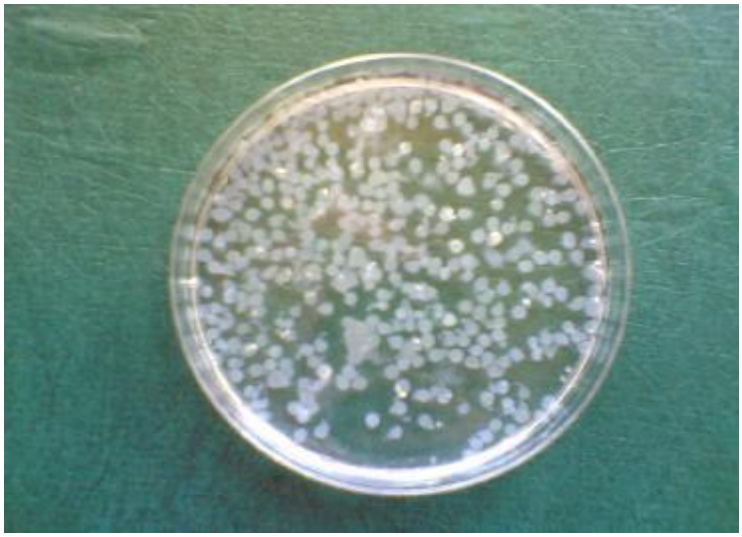

**Figure 2** Immobilized cysteine proteinase of *E.histolytica* by Ca-alginate

**3- Factors influence the activity of free and immobilized cystein proteinase**

**1-pH**

The effect of different level of pH values on the activity of free (mobilized) and immobilized cysteine proteinase produced from trophozoite of *E. histolytica* is shown in figure (3). The highest activity for free enzyme was 69 u/ml at pH 7.5 and then began to decrease until reach to 6 u/ml at pH 4.5 and 9 u/ml at pH 11.5, while the immobilized enzyme cysteine proteinase reached its maximum value 71 u/ml at pH 7.5. Enzyme activity remain stable when pH value is changed to 6.5, 8.5 and 9.5 where as its activity were (68, 71 and 67) u/ml respectively then began to decrease until reach to 19 and 20 u/ml at pH 4.5 and 11.5 respectivily .

A previous Study done by [16] studied the effect of changing in pH value on the activity of free and immobilized protease being isolated from *Aspergillus oryzae* and they noticed that free protease was stable at the pH range 7.0-9.0, where as the immobilized protease was stable over a wider range of pH (5.5 – 10.0) even at pH 10.5, the immobilized enzyme could retain 80% of activity while free enzyme was totally inactivated in this pH. [11] pointed out that the best pH for activity of free aminopeptidase extracted from chicken intestine was 7.0, while in immobilized enzyme reached to pH 9.

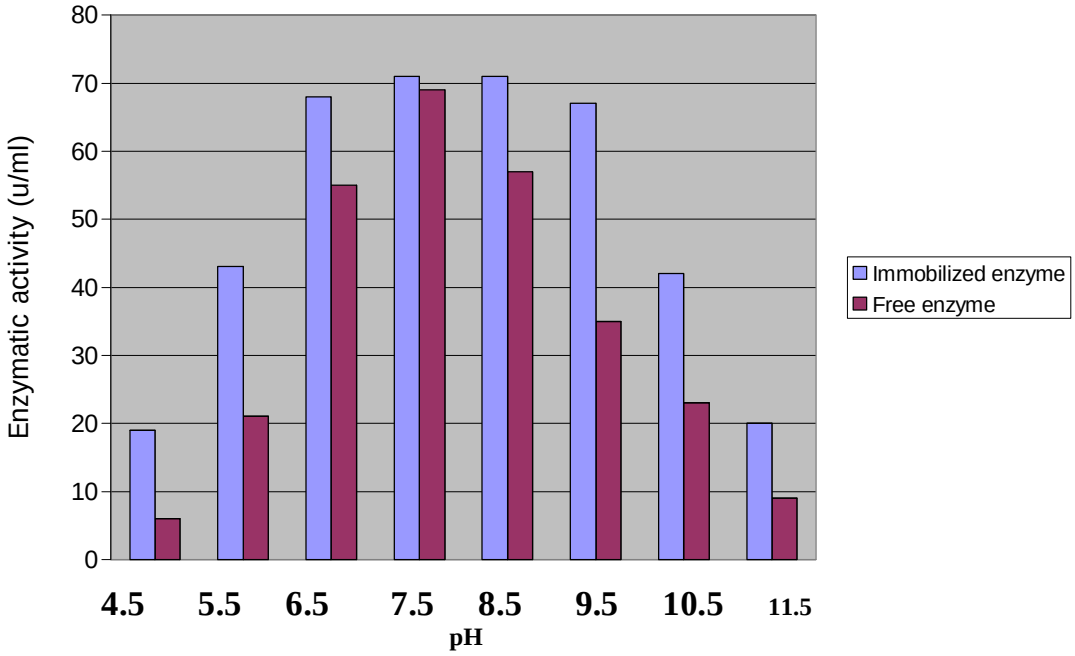


**Figure 3.** Effect of pH on the activity of free and immobilized cysteine proteinase of *E.histolytica*

**2-Temperature**

The enzymatic activity of cysteine proteinase produced by *E. histolytica* was studied by incubation at various temperature degrees. As shown in figure (4). The free and immobilized enzyme reached maximum activity 69 u/ml and 71 u/m at 37Cº.

Temperature above and below 37Cº effectively decreased the enzymatic activity of free enzyme, it

was (16, 58, 69 and 38) u/ml at (30, 35, 37 and 40) Cº respectively. Meanwhile the effect of temperature on enzymatic activity of immobilized enzyme was very little, enzymatic activity was (53, 70, 71 and 71) u/ml at the same temperature degrees. But free and immobilized enzyme decreased to 5 and 10 u/ml at low temperature (20Cº) and to 6 and 30 u/ml at high temperature 50 Cº. This results show the immobilized enzyme with Ca-alginate have ability to retain its activity at various range of temperature, comparing to free enzyme (non- immobilized enzyme), it may be atributed to the fact that Ca- alginate has protected to bounded enzyme from effected harmful temperature. The results of this study were consistent with results of Al-Khafaji (2006) and [11] whose pointed that the immobilized enzyme preserves its activity with different temperature.

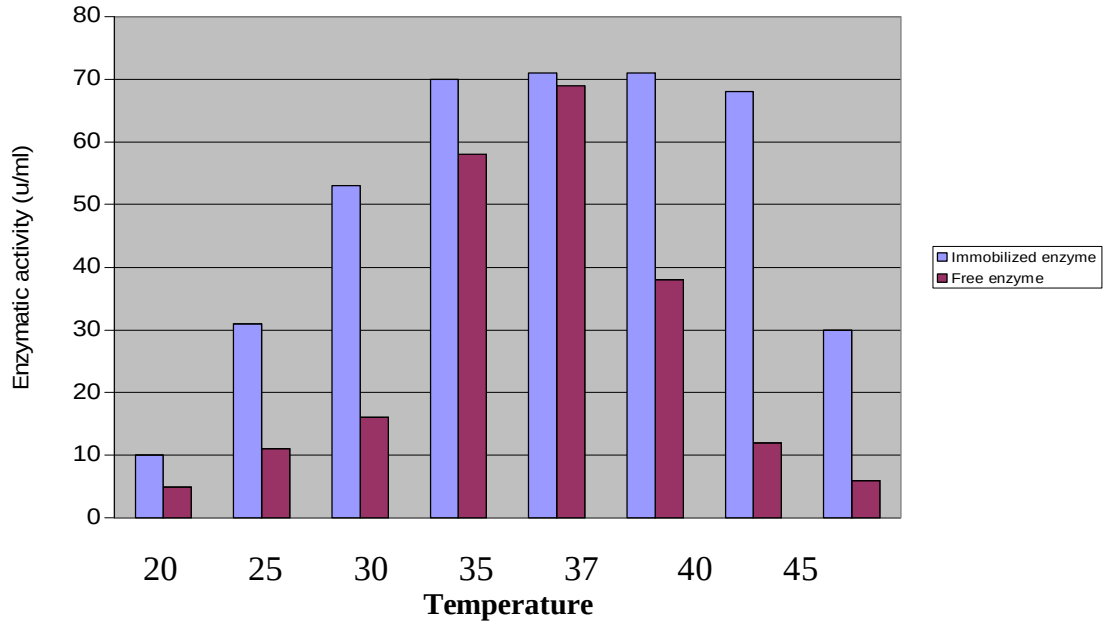


**Figure 4**. Effect of temperature on the activity of free and immobilized cysteine proteinase

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