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Purification and Characterization of Fibrinolytic Enzyme Produced by *Bacillus subtilis* Egy.

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ABSTRACT

A novel extracellular enzyme with strong fibrinolytic activity, produced by *Bacillus subtilis* Egy, which was isolated from the Egyptian soil was purified and characterized. The enzyme was secreted by cultured *B.* subtilis Egy under solid state fermentation and purified 21.5 purification fold using precipitation by ammonium sulphate followed by ion exchange chromatography, and gel filtration on Sephadex G- 100 chromatography. Purified enzyme showed optimum activity at 50^{o}Creaction temperature and pH 8. Enzyme stability studies revealed that the enzymeis stable up to 30 ℃ and it retained 80% of its activity at 40^{o}C.In addition, the purified enzyme is stable at pH 8-9. The fibrinolytic activity was enhanced by Mn^{2+},Cu^{2+}, Ca^{2+}, Na^{+}, and Ba^{2+} and inhibited by, Zn^{2+}, and Hg^{2+}. Moreover, the activity was decreased by EDTA and PMSF at 2 mM final concentration.

INTRODUCTION

Heart attack is medically termed as Myocardial infarction. It happens when blood stops flowing properly to part of the heart. Formation of the blood clot may sometimes block the blood flow causing myocardial infarction and stroke (Deepak *et al.*, 2010).

Fibrin is the main protein component of the blood clot, and it is normally formed from fibrinogen by the action of thrombin (Xiao, *et al*., 2004; Yong *et al*., 2001). Equilibrium in clot formation and its dissolution is necessary in order to maintain the blood flow correctly. (Bajzar, *et al*., 1996). Fibrinolytic therapy is used to achieve the most important objective in patients with an acute myocardial infarction, to open quickly the coronary flow after the occurrence of an acute coronary occlusion. At this time, the external supplement will be very much useful in saving an individual from mortality and morbidity,(Joeng *et al*., 2007).

Microbial fibrinolytic enzymes are agents that dissolve fibrin clots and haveconsiderable potential to be developed into thrombolytic agents (peng, *et al*., 2005). The fibrinolytic enzymes were successively discovered from different microorganisms, the most important among is the genus *Bacillus* from traditional fermented foods (Batomunkueva and Egorov, 2001; Tough, 2005).

Bacillus subtilis produces a variety of extracellular and intracellular proteases including nattokinase (Nakamura, *et al*., 1992). The fibrinolytic enzymes from *Bacillus* spp. have attracted interest as thrombolytic agents because of their efficiency in the fibrinolytic process including plasmin activation.

In the literature, fibrinolytic enzymes were purified by Tien *et al*. (2000) for an enzyme produced by a mutant of *Bacillus subtilis* IMR-NK1, Wang *et al*. (2008) for an enzyme produced by *Bacillus subtilis* LD-8, Cong *et al*. (2009) for an enzyme produced by *Bacillus subtilis* natto B-12, Agrebi *et al*. (2010) of fibrinolytic enzymes by a newly isolated *Bacillus amyloliquefaciens* An6, Deepak *et al*. (2010) by *B. cereus* NK1, Matselyukh *et al*. (2012) by *Bacillus thuringiensis* IMB B-7324, Bajaj *et al*.(2014) by *Bacillus subtilis* I-2, Biji *et al*. (2016) by *Bacillus cereus* IND5, and more recently by Xin *et al*., (2018) by *Bacillus tequilensis*.

In this study, we report purification and characterization of potent fibrinolytic enzyme produced by a newly isolated *Bacillus subtilis* Egy.

MATERIALS AND METHODS

Substrates Chemicals and Reagents:

Fibrin was obtained from bovine blood from MP Biomedicals, LLC. Germany. L-tyrosine was obtained from BDH. England. DEAE Cellulose was obtained from Pharmacia, Sweden. It was used an ion exchange matrix for enzyme purification.Sephadex, gel filtration material was obtained from pharmacia, Biotech., Uppsala, Sweden. Folin and ciocalteu`s phenol reagent was obtained from BDH limited pool, England.All other chemicals are of analytical grade.

Fibrinolytic Enzyme Production

Fibrinolytic enzyme was produced by solid-state fermentation technique in 250 ml conical flasks. Ten grams of wheat bran with fodder yeast as a nutrient substrate (3.6% final concentration) were sterilized at 121^{o}C for 20 minutes. After inoculation with the newly isolated *Bacillus subtilis* Egy. (moisture content 50%), the flasks were incubated at 30^{o}C for five days. After the incubation period, the enzyme was extracted by weighing one gram of fermented culture in 10 mL of dist. Water and subjected it to shaking at 125 rpm for 1hr. Then the liquid enzyme was centrifuged at 4000 rpm for 20 minutes and the supernatant was used as the crude enzyme.

Assay of Enzyme Activity and Protein Content.

Fibrinolytic activity was determined by using fibrin as the substrate according to the method of Raafat *etal*. (2012) with minor modifications. Assay mixture containing 0.5 mL of appropriately diluted enzyme and 0.5 mL of Tris –HCl buffer (0.2 M, pH 8.0) in a test tube containing 10 mg fibrin was incubated at 40°C for 30 min. The reaction was stopped with 1.0 mL of trichloroacetic acid (TCA, 10%, w/v), and contents were centrifuged (4000 rpm for 10 min at 4°C), and the supernatant was subjected to color reaction using Folinciocalteu" s phenol reagent. Optical Density was measured at 650 nm with L-tyrosine as a standard curve. One unit of enzyme activity was defined as the amount of enzyme required to release 1 µg of L-tyrosine from fibrin per min under assay conditions.

Purification of the Fibrinolytic Enzyme:

Fibrinolytic enzyme produced by *Bacillus subtilis* Egy. By solid-state fermentation was subjected to purification scheme. All steps were conducted under 4^{o}C. The extracted enzyme (1g fermented culture in 10 mL dist. Water) was employed to ammonium sulphate precipitation. The precipitate (30- 60% w/v)formed was centrifuged at 4000 rpm for 20 min and the precipitate obtained was dissolved in minimum amount of 50 mMTris-HCl buffer, pH 8.0. The darkbrown solution thus obtained was dialyzed at 4^{o} C for 24-48 h against 10 mMTris-HCl buffer, pH 8.0.The dialyzed partially purified enzyme was applied to two chromatographic purification

columns namely ion exchange chromatography and gel filtration chromatography.

Ion Exchange Chromatography:

DEAE Cellulose (Pharmacia, Sweden) was used for anion exchange in column (bed dimension 2.4 x 30 cm), which was equilibrated with 10 mMTris-HCl buffer, pH 8.0. The column was injected by the dialyzedammonium sulphate fraction (3 mL/run). Elution was carried out in a step-wise manner at a flow rate of 2 mL/ min at regular intervals (4 min/fraction). The first step of elution was carried out with the column buffer containing 0.1 M NaCl, the second elution was with the same buffer containing 0.5 M NaCl and the third elution was with the same buffer containing 1 M NaCl. All the individual fractions were analyzed for fibrinolytic activity on fibrin as a substrate. The active fractions obtained after anion exchange chromatography was further purified by gel filtration chromatography.

Gel Filtration Chromatography:

Sephadex G- 100 (Pharmacia, Sweden) was used for gel filtration chromatography. A column (2.4 X 45 cm) was equilibrated with 50 mMTris-HCl buffer, pH, 8.0. The active fractionsfrom the first column were injected into this column (3 mL/run) and eluted with the same buffer. Fractions were collected at the flow rate of 2 mL/min at regular intervals (4 min/fraction). Column operation and monitoring were similar to that described under ion-exchange chromatography.

Effects of pH, and Temperature on Enzyme Activity:

The effect of pH on the activity of the purified enzyme was determined at 37^{o}C and various pH values, from 5 to 9. Various buffers at a concentration of 50 mM in the reactions were used namely citric acid buffer (pH 5-6), phosphate buffer (pH 5-7), Tris buffer (pH 7-9), and sodium carbonate buffer (pH 9-12). The effect of temperature on the purified enzyme activity was determined at the temperatures 30, 40, 50, 60, 70, or 80^{o}C in 50 mM Tris- HClbuffer (pH 8).

Effects of pH and Temperature on Enzyme Stability:

The effect of pH on the stability of the purified enzyme solution was carried out by incubating the enzyme solution in 50 mM various buffers with pH from 5 to 9, as described above. The residual fribrinolytic activity was analyzed after 30 min. The thermal stability of the purified enzyme solution was evaluated by measuring the residual enzyme activity after incubation of enzyme solution (in 50 mMTris buffer, pH 8) at various temperatures (20, 30, 40, 50, 60, 70 and 80^{o}C) for 10 min.

Effects of Metal Ions and Inhibitors on Enzyme Activity:

The effects of mono-, di- and trivalent metal ions, and inhibitors on the enzyme activity were carried out by pre- incubating the enzyme solution with the chemicals of KCl, NaCl, $MgCl_2$, $CuSO_4$, $CaCl_2$, $ZnCl_2$ and $FeCl_3$, and the inhibitors of EDTA, L-cysteine, PMSF and 2-Mercabtoethanol at a concentration of 2 mM in 50 mM Tris-HCl buffer (pH 8.0) at 40^{o}C for 30 min. The fibrinolytic activity of the enzyme solution at each incubation condition was assayed. The relative activity was expressed as a percentage of the original enzyme activity without any effectors.

Determination Soluble Protein:

Throughout this work, the soluble protein of crude enzyme preparation was determined according to Ohnistti and Barr (1978).

The protein in the purified enzyme was determined by U.V. absorbance technique at 280nm.

RESULTS AND DISCUSSION

Purification of Fibrinolytic Enzyme Produced by *Bacillus subtilis* Egy.

Three-step purification scheme was applied to purify the enzyme under study. The crude culture extract of the bacterial culture on 3.6% fodder yeast under solid-state fermentation conditions was firstly partially purified by salting out with ammonium sulphate (30-60% saturation). Under these conditions, specificactivity

reached 231.7 U/mg protein and yield recovery 35.6%.

After dialysis against 50 mM Tris-HCl buffer pH 8.0 overnight, this partially purified enzyme was applied to the ion exchange column on DEAE cellulose. 45 fractions were collected using Tris-HCl buffer, sodium chloride eluting solution. The results are graphically illustrated in Fig (1): The enzyme was successfully eluted at fraction 11 &12. These two fractions were collected from several runs, pooled and applied to the third purification step (Gel filtration on Sephadex G-100). As shown in Fig (2), the enzyme was effectively eluted in fraction 9 and 10 with specific activity of 1270 U/mg protein and yield recovery of 5.26%. The overall purification scheme, purification steps, purification folds and percentage of yield recovery are presented in Table (1). Accordingly, the fibrinolytic enzyme of *Bacillus subtilis* Egy. was purified 21.5 times with final yield recovery of 5.26%. Cong *et al.*(2009) produced nattokinase from isolated *Bacillus subtilis* nattoB-12, the enzyme was purified to 56.1-fold, with a recovery of 43.2% of the initial activity. Kotb (2014) reported that purification scheme increased the fibrinolytic activity 443.5-fold with a yield recovery of 17%. Bajaj *et al.*(2014) studied the production of fibrinolytic protease from *Bacillus subtilis* I-2. Ammonium sulfate precipitation and DEAE Sephadex chromatography resulted in 4.8-fold purification of protease.

Table 1: Purification scheme, steps and yield recoveryof the fibrinolytic enzyme produced by *Bacillus Subtilis* Egy.

Purification Steps	Total Activity(U)	Total Protein(mg)	Specific Activity(U/mgprotein)	Yield %	Purification Fold
Crude culture filtrate	2050	34.8	58.9	100	1
30-60% Amm.sulphate	730	3.15	231.7	35.6	3.93
DEAG-cellulose	141	0.35	402	6.87	6.82
Sephadex G-100	108	0.085	1270	5.26	21.5

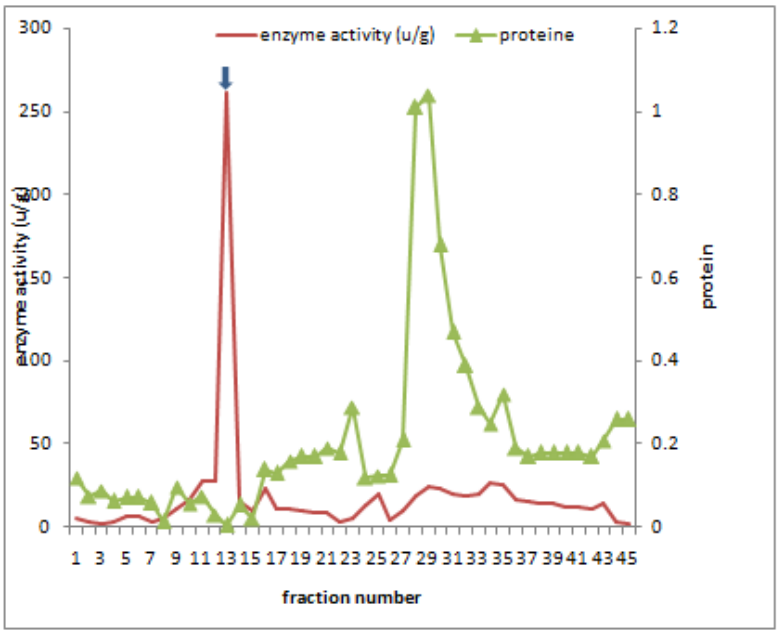

Fig. 1: Ion exchange chromatography of amm. sulphate precipitated fraction (30-60%) fibrinolytic enzyme of *Bacillus subtilis* Egy. on DEAE cellulose (The black arrow points at the peak of fractions with fibrinolytic activity).

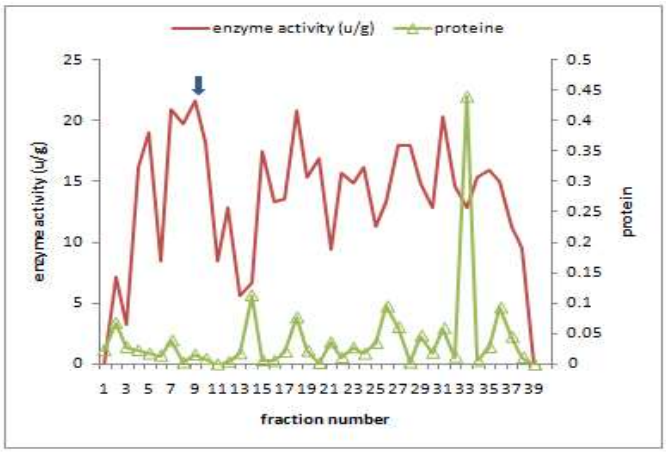

Fig. 2: Gel filtration of fractions (11, 12) of ion exchange chromatography on Sephadex G-100. (The black arrow points at the peak of fractions with fibrinolytic activity).

Properties and Kinetics of Purified Fibrinolytic Enzyme of *Bacillus subtilis* Egy.

Temperature Profile of Purified Fibriolytic Enzyme

Thermal stability and effect of different reaction temperatures are presented in Fig (3).

The fibrinolytic activity of the purified enzyme of *Bacillus subtilis* Egy after heating in water bath sets at different temperatures was recorded.

It is clear that the purified enzyme is heat stable upon heating to 30°C without apparent loss of activity. However, heating the enzyme at 60°C for 15 minutes drastically reduced the activity whereas total denaturation of the enzyme was evident at higher temperatures.

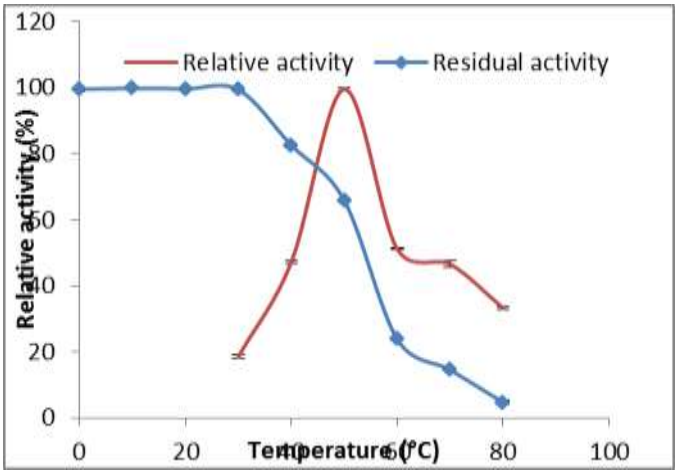

Fig. 3: Thermal stability and effect of different incubation temperatures on activity of purified fibrinolytic enzyme.

On the other hand the fibrinolytic activity of the purified enzyme of *Bacillus subtilis* Egy was measured at different reaction temperatures between 20°C and 80°C under standard reaction conditions. The results revealed that purified enzyme activity increased progressively with incubation reaction temperature up to 50°C. At higher reaction temperatures, a notable decrease in enzyme activity was evident. Kotb (2014) found that maximal activity was attained at a temperature of 40°C while, Wang *et al.*

(2008) reported that the optimal temperature of fibrinolytic enzyme was 50°C Cong *et al*. (2009) Biji *et al*. (2016) also purified the fibrinolytic enzyme that was highly active at 50°C, which agrees with our results. Vijayara-ghavan *et al*. (2016) reported that the fibrinolytic enzyme optimal temperature was 60°C and was stable up to 50°C. Matselyukh *et al*. (2012) purified fibrinolytic enzyme of *Bacillus thuringiensis* IMB B-7324 with optimal temperature was 50°C.

Effect of pH Value and pH Stability on Purified Fibrinolytic Enzyme Activity.

Fig. 4: shows the enzyme activity detected upon buffering reaction mixture with phosphate, Tris-HCl and Carbonate - bicarbonate buffers at pH range between 5.0 to 10.0. Our results recorded that the highest fibrinolytic activity takes place at pH 8.0. The results also showed that the purified enzyme is pH stable in the range ofpH 8-9 while, less residual activity is recorded at pH 10. Kotb (2014) found that maximal activity was attained at a pH of 8.0, the same reported by Wang *et al*. (2008). Biji *et al*. (2016) investigated that purified fibrinolytic enzyme was stable at pH 8.0, the previous studies are in agreement with our results. Vijayara-ghavan *et al*. (2016) reported that the fibrinolytic enzyme depicted an optimal pH of 9.0 and was stable at a range of pH from 8.0 to 10.0.

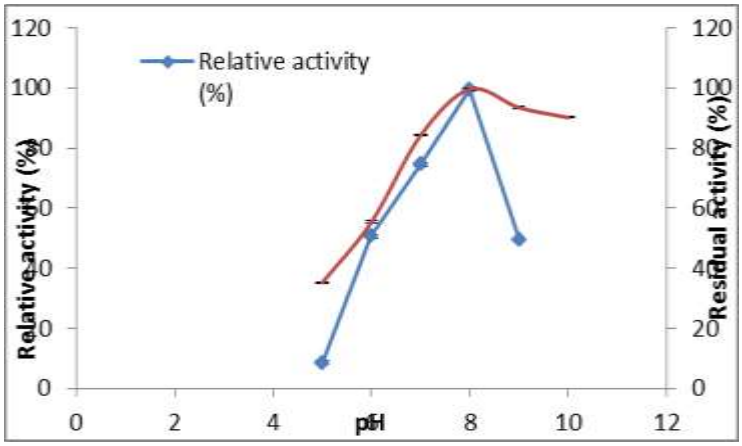

Fig. 4: Effect of pH value and pH stability on purified fibriolytic enzyme activity.

Effect of Incubation Time on the Activity of Purified Enzyme.

Fig. 5: shows the effect of different reaction incubation times on purified enzyme activity from 10 to 60 min. The results indicated that the purified enzyme activity is increasing with increase the reaction incubation time till 60 min.

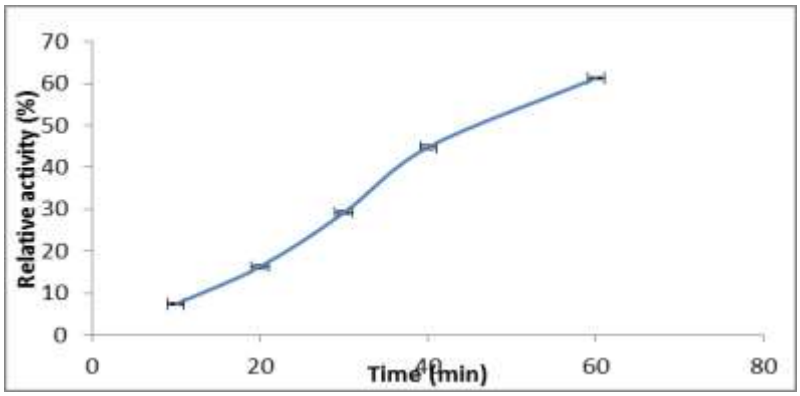

Fig. 5: Effect of incubation time on the activity of purified enzyme activity.

Effect of Substrate Concentration on the Activity of Purified Enzyme Activity. Different concentrations of fibrin were incorporated into the standard reaction mixtures in the range from 2 to 20 mg/mL. The results are graphically illustrated in Fig. **(6)**. It is clear that the enzyme activity responded linearly to the increase in substrate concentration up to 8 mg/mL reaction mixture. While, at higher fibrin concentrations linearity was not presented.

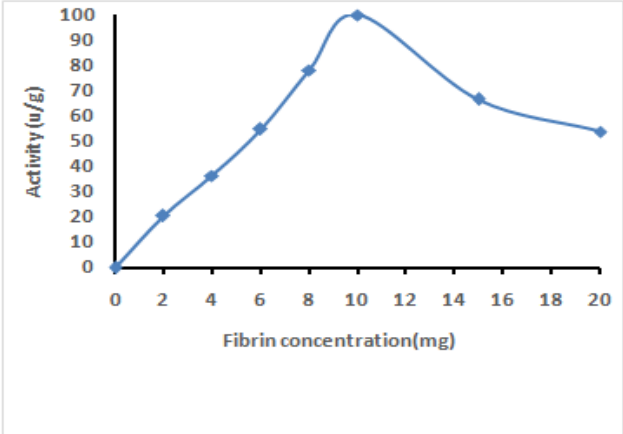

Fig. 6: Effect of substrate concentration on the activity of purified enzyme activity.

The Km value of the purified enzyme for fibrin as a substrate was calculated to be 3.7 mg/mL as shown in Fig (7).Tien *et al*., (2000) reported a $K_m$of 0.15% for fibrin hydrolysis.

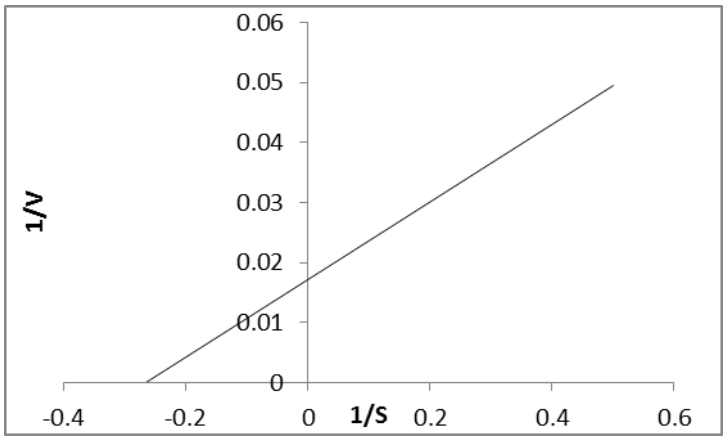

Fig. 7: Line weaver-Burk plot of the reciprocals of the initial velocities and fibrin concentration.

Effect of Different Metal Ions on the Activity of Purified Fibrinolytic Enzyme of *Bacillus subtilis* Egy.

In this experiment, a number of metal salts were incorporated into the standard reaction mixtures containing the purified enzyme. The metal ions were tested at 2mM final concentration. The results are graphically illustrated in Fig. (8).

The obtained results indicated that some metal ions increase the activity of purified enzyme such as $Mncl_2$, $CuSo_4$, $CaCl_2$, and NaCl whereas some salts inhibited the enzyme activity such as $ZnCl_2$ and $HgCl_2$.

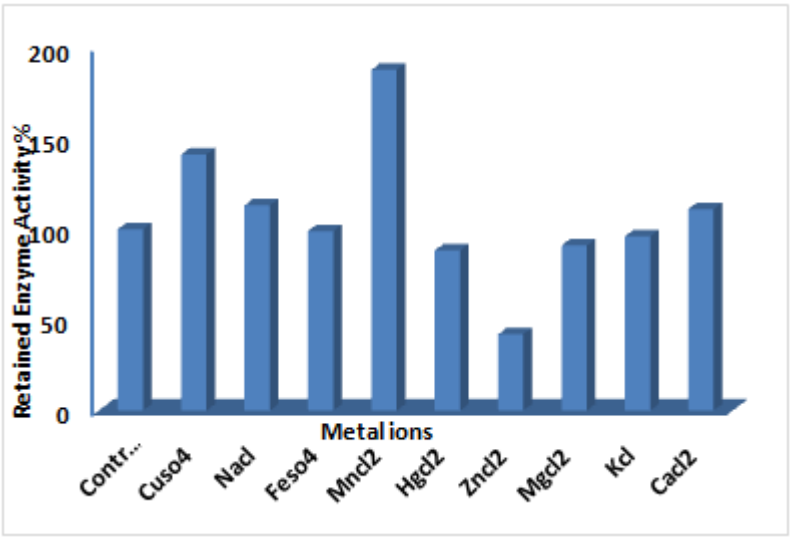

Fig. 8: Effect of different metal ions on the activity of purified fibrinolytic enzyme of *Bacillus subtilis* Egy.

Effect of Some Inhibitors or Additives on the Activity of Fibrinolytic Enzyme.

The results in Table (2) indicated that significant inhibition of enzyme activity was observed when PSMF and EDTA were separately incubated with the purified enzyme. The result indicated that the purified enzyme is a serine metallo- protease.

Table 2: Effect of some inhibitors on the activity of the fibrinolytic enzyme.

Inhibitor or Additives	Retained enzyme activity (%)
Non	**100**
EDTA	**51.8**
PSMF	**79.9**

REFERENCES

Agrebi, R.; Hmidet, N.; Hajji, M.; Ktari, N.; Haddar, A.; Fakhfakh-Zouari, N.; Nasri, M. (2010): Fibrinolytic serine protease isolation from *Bacillus amyloliquefaciens* An6 grown on Mirabilis jalapa tuber powders. Applied Biochemistry and Biotechnology. 162(1):75-88.

Bajaj, B. K.; Satbir Singh; Mowkshi Khullar; Kaurab Singh; Shikha Bhardwaj (2014): Optimization of fibrinolytic protease production from *Bacillus subtilis* I-2 using agro-residues. Brazilian Archives of Biology and Technology. 57(5):653-662. 30.

Bajzar, L., Nesheim, M. E. and Tracy, P. B. (1996): The profibrinolytic effect of activated protein C in clots formed from plasma is TAFI-dependent. Blood, 88 (6): 2093–2100.

Batomunkueva, B. P. &Egorov, N. S. (2001): Isolation, purification and resolution of the extracellular proteinase complex of *Aspergillusochraceus* 513 with fibrinolytic and anticoagulant activities. Microbiology, 70(5): 519-522.

Biji, G. D.; Arumugaperumal Arun; EswaranMuthulakshmi; PonnuswamyVijayaraghavan; Arasu, M. V.; Al-Dhabi, N. A. (2016): Bio-prospecting of cuttle fish waste and cow dung for the production of fibrinolytic enzyme from *Bacillus cereus* IND5 in solid state fermentation. 3 Biotech. 6(2): 231. 66.

Cong W., Ming D., Mei Z.D., Dong K.F. Ren Z.G., Bing F. Y. (2009).

Purification and characterization of nattokinase from *Bacillus subtilis natto*B- 12. Journal of Agricultural and Food Chemistry 57 (20): 9722 – 9729.

Deepak V., Ilangovan, S., Sampathkumar, M. V., Victoria, M. J., Pasha, S. P. B. S.,Pandian, S. B. R. K., *et al*. (2010): Medium optimization and immobilization of purified fibrinolytic URAK from *Bacillus cereus* NK1 On PHB nanoparticles. Enzyme and Microbial Technology, 47(6): 297–304.

Deepak V., Ilangovan, S., Sampathkumar, M. V.,Victoria, M. J., Pasha, S. P. B. S.,Pandian, S. B. R. K., *et al*. (2010): Medium optimization and immobilization of purified fibrinolytic URAK from *Bacillus cereus* NK1 On PHB nanoparticles. Enzyme and Microbial Technology, 47(6): 297–304.

Joeng, S. J., Kown, G. H., Chun, J., Kim, J. S., Park, C. H., Kwon, D. Y., *et al*. (2007): Cloning Of fibirinolytic enzyme gene from *Bacillus Subtilus* isolated from Cheong gukjang and its expression in protease deficient *Bacillus subtilus* strain. Journal of Microbiology and Biotechnology, 17(6): 1018–1023.

Kotb, E. (2014): Purification and partial characterization of a chymotrypsin-like serine fibrinolytic enzyme from *Bacillus amyloliquefaciens* FCF-11 using corn husk as a novel substrate. World Journal of Microbiology & Biotechnology. 30(7): 2071-2080. 41.

Matselyukh, O. V.; Nidialkova, N. A.; Varbanets, L. D. (2012): Purification and physico-chemical properties of *Bacillus thuringiensis* IMB B-7324 peptidase with elastolytic and fibrinolytic activity. [Ukrainian] Ukrainskii Biokhimicheskii Zhurnal. 84(6): 25-36. 23.

Nakamura, T., Yamagata, Y. & Ichishima, E. (1992): Nucleotide sequence of the sub tilisin NAT gene, aprN of *Bacillus subtilis* (natto). Bioscience, Biotechnology and Biochemistry, 56: 1869–1871.

Ohnisitti S. T. and Bar J. K. (1978): A simplified method for quantitating protein. The biuret and phenol reagents.Anal Biochem.86: 193- 200.

Peng, Y., Yang, X. & Zhang, Y. (2005): Microbial fibrinolytic enzymes: An overview of source, production, properties, and thrombolytic activity in vivo. Applied Microbiology and Biotechnology, 69: 126–132.

Raafat A., Araby E and Lotfy S. (2012): Enhancement of fibrinolytic enzyme production from *Bacillus subtilis*via immobilization process onto radiation synthesized starch / dimethylaminoethyl methacrylate hydrogel Carbohydrate polymers 87: 1369 – 1374.

Tien C. C; Ming Hui F; Chi K. F; Yi S. H. (2000): Potent fibrinolytic enzyme from a mutant of *Bacillus subtilis* IMR-NK1. Journal of Agricultural and Food Chemistry; 48(8): 3210-3216. 34 ref.

Tough, J. (2005): Thrombolytic therapy in acute myocardial infarction. Nursing Standard, 19(37): 55–64.

Vijayaraghavan P; Vincent, S. G. P.; Arasu, M. V.; Al-Dhabi, N.A. (2016): Bioconversion of agro-industrial wastes for the production of fibrinolytic enzyme from *Bacillus halodurans* IND18: purification and biochemical characterization. Electronic Journal of Biotechnology; 2016. 20:1-8.

Wang Cong; Du Ming; Zheng DongMei; Kong FanDong; Zu GuoRen; Feng YiBing (2009): Purification and characterization of nattokinase from *Bacillus subtilis* natto B-12. Journal of Agricultural and Food Chemistry; 57(20): 9722-9729.

Wang, S. H.; Zhang Cheng; Yang, Y. L.; Diao Miao; Bai, M. F. (2008): Screening of a high fibrinolytic enzyme producing strain and characterization of the fibrinolytic enzyme produced from *bacillus subtilis* LD-8547. World Journal of Microbiology & Biotechnology, 24(4):475-482. 21.

Xiao, L., Zhang, R-H., Peng,Y. and Zhang, Y-Z. (2004): Highly efficient gene

expression of a fibirinolytic enzyme (subtilisin DFE) In *Bacillus subtilus* mediated by the promoter of alpha-amylase gene from *Bacillus amyloliquefaciens*. Biotechnology Letters, 26, 1365–1369.

Xin, Xiong, Ambati R.R., Cai Z., Lei B. (2018): Purification and characterization of fibrinolytic enzyme from a bacterium isolated from soil. 3Biotech February 2018, 8:90.

Yong K. J., Jeong U.P., Hyun B., Sung H.P., In Soo K., Dong W.K et al .(2001): Purification and biochemical characterization of a fibrinolytic enzyme from *Bacillus subtilis* BK- 17 . World Journal of Microbiology and Biotechnology , 17 :89- 92.

ABIC SUMMARY

تنقية ودراسة خواص الانزيم المحلل للفبرين المنتج بواسطة يكتيرة *باسلس ستلس* ايجى

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تم انتاج انزيم خارج الخلايا له نشاط محلل للفبرين بواسطة يكتيرة *باسلس ستلس* ايجى وهى معزولة من التربة المصرية. تم انتاج الانزيم بتطبيق التنمية بالمنابت الصلبة. اجريت لهذا الانزيم عملية تنقية من خلال ثلاث خطواتهى الترسيب باستخدام ملح كبريتات الامونيوم ثم عمود كروماتوجرافى تبادل ايونات تلاه عمود كروماتوجرفى فلترة الجيل سيفادكس جى ١٠٠ . من خلال هذه الخطوات تم تنقية الانزيم ٢١،٥ مقارنة بالانزيم الخام. وبدراسة خواص الانزيم المنقى وجد ان درجة الحرارة المثلى لنشاطه الانزيمى هى ٥٠ درجة مئوية وانه ثابت حراريا لدرجة ٣٠ درجة مئوية فى حين انه يحتفظ ب ٨٠ % من نشاطه الانزيمى عند درجة ٤٠ درجة مئوية.اما درجة الاس الهيدروجينى المثلى لنشاطه فهى ٨ اما عن ثباته لدرجات الاس الهيدروجينى المختلفة فهو ثابت عند درجة اس هيدروجينى ٨- ٩ اما عند ١٠ درجة اس هيدروجينى فانه يحتفظ ب ٩٠% من نشاطه الانزيمى. وبدراسة تاثير وقت التفاعل على النشاط الانزيمى للانزيم المنقى وجد انه يزداد بزيادة زمن التفاعل الى ٦٠ دقيقة. كما وجد ان انسب تركيز للفبرين فى التفاعل الانزيمى هو ١٠ ملى جرام/ ملوبحساب قيمة ثابت ميكالس للتفاعل وجد انه ٧،٣ ملى جرام/ مل. كما تم دراسة تاثير بعض الايونات المعدنية على النشاط الانزيمىللانزيم المنقى حيث كان لكل من ايونات المنجنيز والنحاس والكالسيوم والصوديوم تاثير محفز للنشاط الانزيمى بينما ادى وجود ٢ ملى مول من كل من الزنك والزئبق الى تثبيط النشاط الانزيم واخيرا تم استنتاج ان هذا الانزيم ينتمى لمجموعة البروتيز المعدنى لنقص نشاطه عند اضافة الاديتا كما تم استنتاج انه سيرين بروتيزلنص نشاطه باستخدام بى ام اس اب فى التفاعل.