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PURIFICATION AND SOME PROPERTIES OF URICASE FROM *STREPTOMYCES ALBONIGER* ISOLATED FROM EGYPTIAN FODDER CHICKEN

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ABSTRACT

Purification of pure uricase enzyme of *Streptomyces alboniger* was carried out by Gel filtration using Sephadex G-200 column chromatography and agarose gel electrophoresis. The purification process resulted in pure protein preparation with specific activity of 2.2 units/mg protein, thus the purification process increased the concentration up to 550 fold. The molecular weight of pure uricase was 130,000 dalton as determined by polacrylamid gel electrophoresis. The purified uricase showed high activity at pH 9 and 30°C. Also highest activity of pure enzyme was obtained after gamma irradiation at 0.5KGy, and complete inhibition of the enzyme activity occurred at 3 KGy.

INTRODUCTION

The production of various degradative enzymes had been reported from members of the genus *Streptomyces*. Some model examples of these enzymes included α-amylase[1]; B-amylase[2]; chitinase[3]; pectinase[4,5]; cellulase[5] proteases and uricase[8].

Uricase, Urate: oxygen oxidoreductase, has been localized intracellularly within a crystalloid structure of the microbiodies. The unit of uricase is the amount of uricase that converts one micromole of uric acid per minute. The properties of this enzyme in solution and the regulation of its activity were previously studied. Other workers suggested that the purified uricase would have some medical importance in the field of chemotherapy[11].

Microbial uricase has been obtained in a highly purified form by workers[8,10-14].

In General, the uricase found in microorganisms is an intracellular enzyme and hence extraction and purification of the enzyme can only be performed after disruption of cells by means of the ultrasonic or freezing and thawing method[15]. The difficuly in purifiying uricase was largely due to a close association of the enzyme with certain nucleoprotein fragments. These aggregates tend to precipitate during the course of the purification procedure. Best results in separating uricase from these impuritis were also previously achieved.

Manwhile, previous purification of the enzyme by gel-filteration through a series of Sephadex G-200 column was tested by agarose gel electrophoreses. A specific activity of 0.144 units/mg protein 288-fold increase in concentration were obtained.

Factors affecting the activity of the purified uricase such as effect of pH and temperature were also previously studied[12,16]. Enzyme deactivation was considered as one of the direct causes of cell death as a result of radiation exposure, thus a lethal irradiation dose caused complete deactivation of all the examined enzyme[17].

MATERIAL AND METHODS

1- Microorganisms:

Streptomyces alboniger Strain was previously isolated from Egyptian fodder chicken[18], its streptomycetal mat was used for extraction and purification of intracellular uricase enzyme.

2- Collection of the mat:

The mats of ten liters of culture in uric acid liquid production medium (starch-nitrate liquid medium) were collected, washed with sterile borate buffer pH 9.0 for several times and the net weight of the fresh mycelia was determined.

3- Extraction of the enzyme from the mat:

The stroptomycetal mat was ground with pure and clean sand beads under cold conditions, using small aliquots of borate buffer at pH 9.0 and centrifuged at 3000 r.p.m. for 30 min. The actual volume of clear supernatant obtained at the end of centrifugation was determined, tested for both enzyme activity and protein content; according to previous methods

The purification streps of the intracellular uricase from *Strep. alboniger* was conducted according to previous and were summarized as following.

A known weight (35 mg) of fresh mycelial mat was obtained by centrifugation from 10 litres culture in uric acid production medium. The mat was washed 3 times using borate buffer pH 9, ground in a mortar with fine sand and borate buffer, then centrifuged. The specific activity of cell free supernatant was determined and protein was collected by centrifugation after precipitation with 60% ammonium sulphate.

The precipitate was dissolved in borate buffer (pH 9) and transferred into plastic bags for dialysis against sugar crystals.

Fractionation was carried out by using a Sephadex column G-200, 100 fractions 10 ml each were collected. The previous processes were repeated again; 2nd ammonium sulphate precipitation, 2nd dialysis against borate buffer pH 9 and fractionation by column of Sephadex G-200, 36 fractions 5 ml each aliquots were obtained and only one sharp peak was taken in consideration.

5- Simple-Agraose Electrophoresis:

A Check for the enzyme purity was carried out as describted by El-Hawary and Ibrahim (1968).

6- Factors affecting activity of uricase:

Determination of Uricase activity under different incubation temperature was carried out according to previous method[20]. Effect of pH values on stability of uricase enzyme was studied. Also the effect of γ-ray on the uricase activity was measured according to Tohamy, (1991)[21].

RESULTS

The aim of the purification procedure was to increase the specific catalytic activity (activity per unit weight) of the enzyme during each purification step. The gneral steps in purification method usually were cell breakage, fractionation of hte cellular homogenate by applying simple gross fractionation, and finally the application of the more selective techniques such as column chromatography.

The relation between specific activity and protein content is presented (Fig. 1) as four peaks from (10 to 36) with specific activity 0.23 unit/mg protein. A second run of purification on Sephadex G-200 resulted in 36 fractions with only one sharp peak with highest specific activity 2.2 unit/mg protein (Fig. 2).

The results in table 1 indicated that the first precipitation with 60% ammonium sulphate increased the specific activity of the enzyme from 0.004 to 0.03 (units/mg protein) and concentration increased up to 12.5 folds (units/mg protein). The second steps of dialysis increased the specific activity from 0.05 to 0.064 (units/ mg protein) and the concentration increased up to 16 folds.

The first application as Sephadex G-200 resulted in specific activity of 0.23 units/mg protein which is corresponding to 57.5 folds. A second purification on Sephadex G-200 increased the specific activity up to 2.2 units/mg protein which is corresponding to 550 folds.

A simple agarose gel electrophoresis was also applied to support the purity of uricase. Polyacrylamide gel electrophoresis was considered for enzyme purity. It was used for the determination of the molecular weight, which was found to be 130,000 Dalton.

After isolating the purified enzyme, further work was directed for studying certain factors affecting activity viz.: effect of temperature, hydrogen ion concentration and effect of gamma radiation.

The results represented in Fig. 3, indicated that the optimum pre-temperature for the activity of purified uricase was 30°C, while the pre-temperature of 0, 60°C caused complete inhibition of uricase.

Table (2): Effect of some metalic salts (50 µg/ml) on the activity of uricase produced by Strep. alboniger.

Different metalic salts (50 µg/ml)	Uricase specific activity (units/mg protein) Strep. alboniger
Control (no metalic ioin:)	0.0010
Magnesium sulphate	0.0050
Zinc sulphate	0.0000
Copper sulphate	0.0054
Ferrous sulphate	0.0045
Cobalt sulphate	0.0068

Also, the results in Fig. 4, showed that the activity of purified uricase was increased gradually with increase in pH where the highest enzyme activity was recorded.

Table (1): Purification of uricase produced by Streptomyces alboniger.

Purification	Volume (ml)	Portein (mg/ml)	Total Protein	Uricase activity (unit/ml)	Total units	Specific activity (units.mg protein)	Purification
1- Cell pre-supernatant	320	20	6400	0.08	25.60	0.004	1
2- 1st amm. msulphate precipitale	90	25	1000	1.25	50	0.05	12.5
3- 1st dialysis prepartion	30	15	450	3.45	103.50	0.064	16
4- Sephadex G-200 column chromatogram	15	10	150	4	60	0.23	57.5
5- 2nd amm. sulphate preciptate	6	5	30	2	12	0.4	100
6- 2nd dialysis preparation	9	3	27	1.38	12.42	0.46	115
7- Sephadex G-200 column chromatogram	9	1.2	10.8	2.64	23.76	2.2	550

The specific activity was dropped by further increase in pH specialy at pH 9.3 and 9.6.

Data in table 2 showed the effect of different metallic ions on the purified uricase of *Strep. Alboniger.* It was clear that zinc sulphate (50 um/ml) was effective inhibitor for the enzyme synthesis. While cobalt sulphate (50 ug/ml) were activators for enzyme followed by copper sulphate.

The results represented in Fig. 5 indicated that the specific activity of the purified enzyme increased gradually by increasing γ-radiation doses from 0 up to 0.5 KGy, then decreased and completely inhibited after exposure to irradiation dose of 3 KGy. The results give an indication about gamma radiation as stimulator for enzyme activity at radiation dose: 0.25, 0.50, 1.0 if compared with non-irradiated one (0.0 KGy).

DISCUSSION

Purification process of uricase produced by *Strep. alboniger* resulted in a pure protein preparation with specific activity of 2.2 units/mg. protein Purification increased the specific activity up to 500 folds.

The results obtained by using polyacrylamide, electrophoresis for determination of the molecular weight showed molecular weight 130,000. This agrees well with the estimate of 120,000 as previously described[22,23] indicating that the native molecular weight of pig liver uricase as estimated by sedimentation equilibrium was 135,000.

The purified enzyme of *Strep. Alboniger* with specific activity of 2.2 units/mg protein was studied further, on sepadex G-200 resulted in a single sharp peak, and agarose-gel electrophoresis resulted in a one protein spot. This supported the degree of purity of enzyme preparation.

The present investigation indicated that the purified uricase was found most active at pH 9. This result agrees with previous data[8,11,16,20,24].

Also, the highest activity was recorded at optimum temperature 30°C for the purified uricase, which agrees with previous results. Moreover, the enzyme was completely inhibited by addition of zinc sulphate at concentration 50 ug/ml and activated by cobalt sulphate, copper sulphate, magnesium sulphate and ferrous sulphate, (50 ug/ml). The inhibitory effect of metal ions was reported before. However stimulatory effect of some metal ions was also reported.

The present investigation revealed that highest activity of the pure enzyme in buffer solution (pH 9) was obtained after irradiation at 0.5 KGy. Since its activity increased by 3.5 folds than the non irradiated one. It also clear that 3 KGy cause complete inhibition of hte enzyme activity.

It was recorded that both the affinity of enzyme-substrate and the reaction velocity of the enzyme substrate complex have changed, while the function of the enzyme is maintained. This suggested that some changes had occurred in the fine structure of the enzyme, without, destroying its catalytic function, although some what modifying. It was also added that the proteins (e.g. enzyme proteins) that have two or even more different biological functions lose only one of the effect of exposure to a given radiation dose, while they invariably retain the other function or functions (IAEA, 1973)[27]. The stimulation of uricase preparation of high purity by irradiation could have a partical importance in the field of chemotherapy. Since reaction against the irradiated and nonirradiated purified uricase when injected intravenously should be tested.

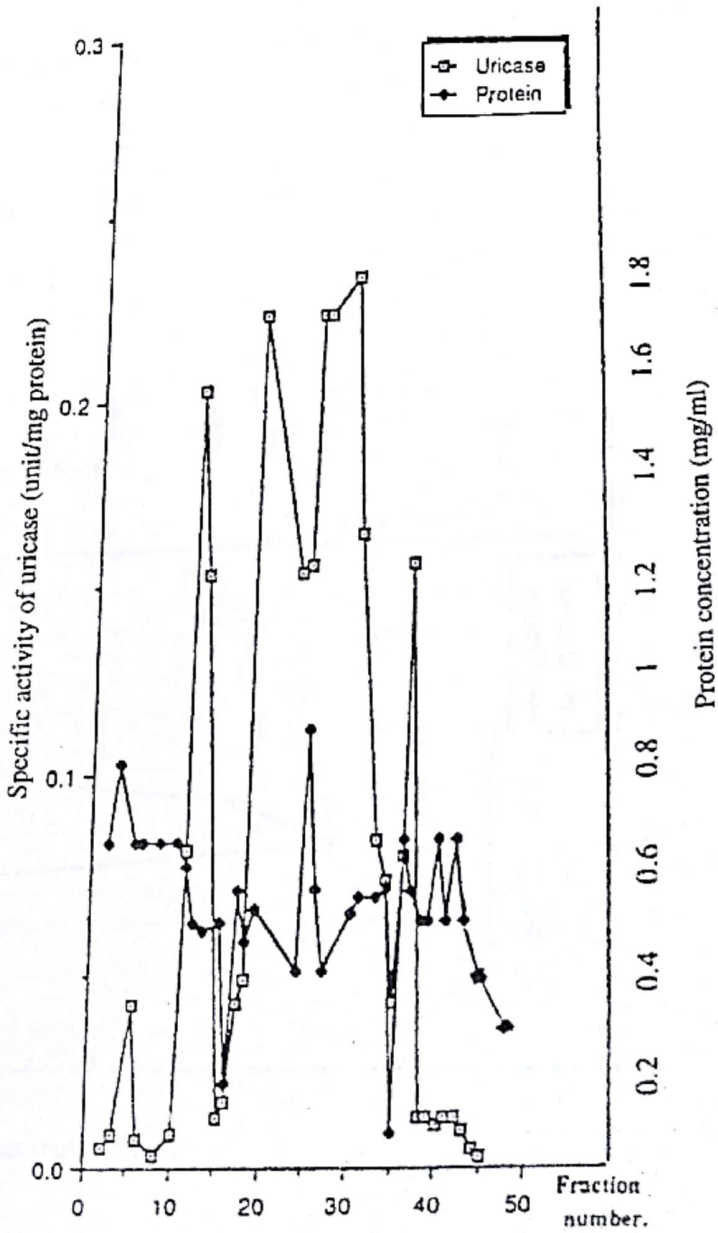

Fig. (1): The specific activity of uricase (unist/mg protein) for each fraction (1-64).

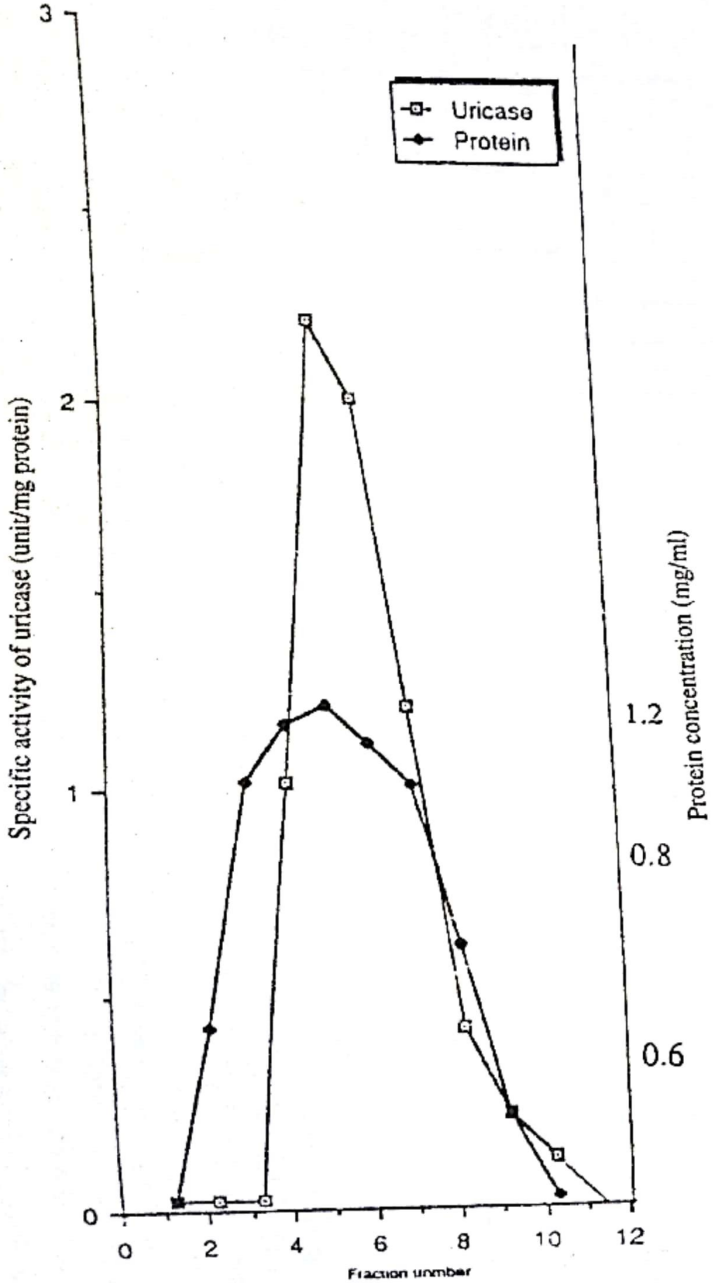

Fig. (2): The specific activity of uricase (unist/mg protein) for each fraction (1-18).

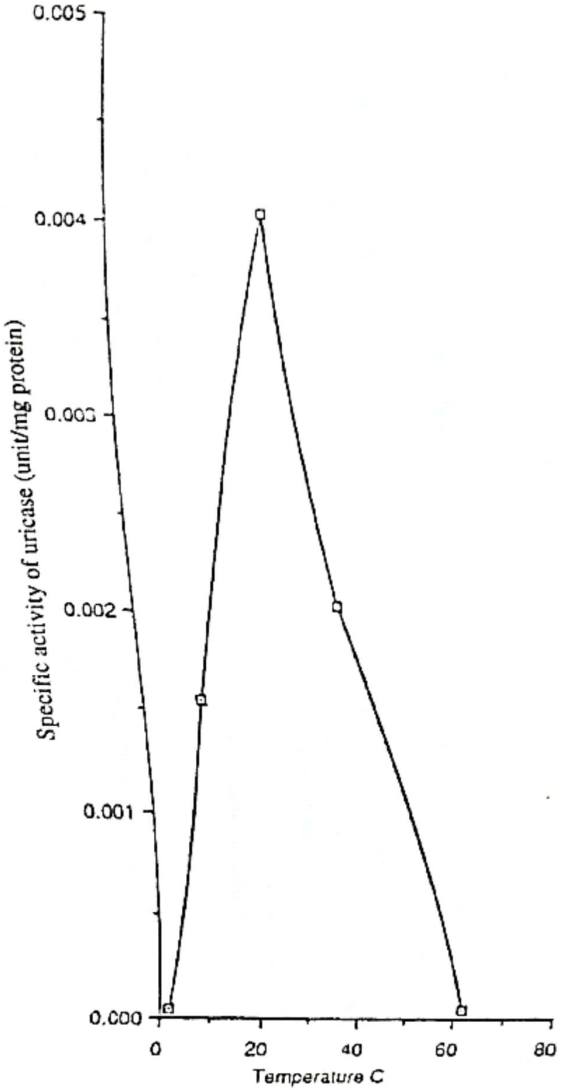

Fig. (3): *Effect of pre-temperature on the purified uricase enzyme produced by Strep. albomiger.*

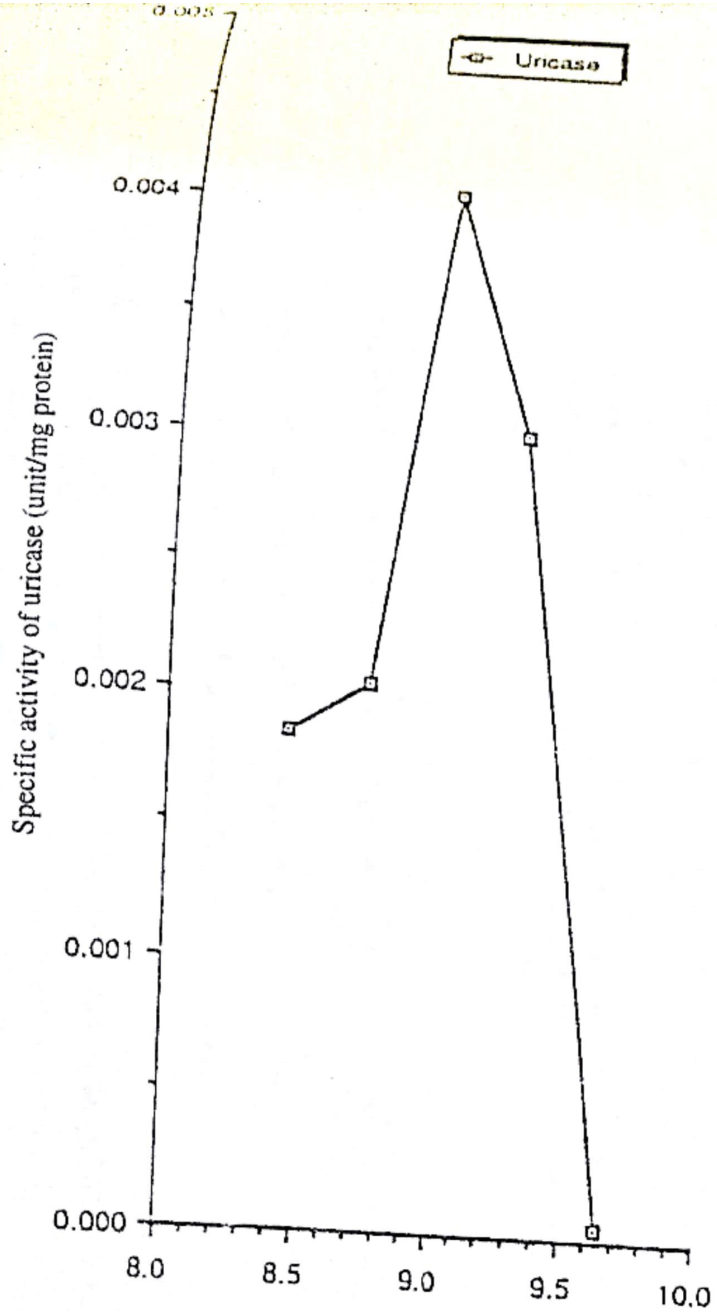

Fig. (4): Effect of pH on the purified uricase enzyme produced by *Strep. alboniger.*

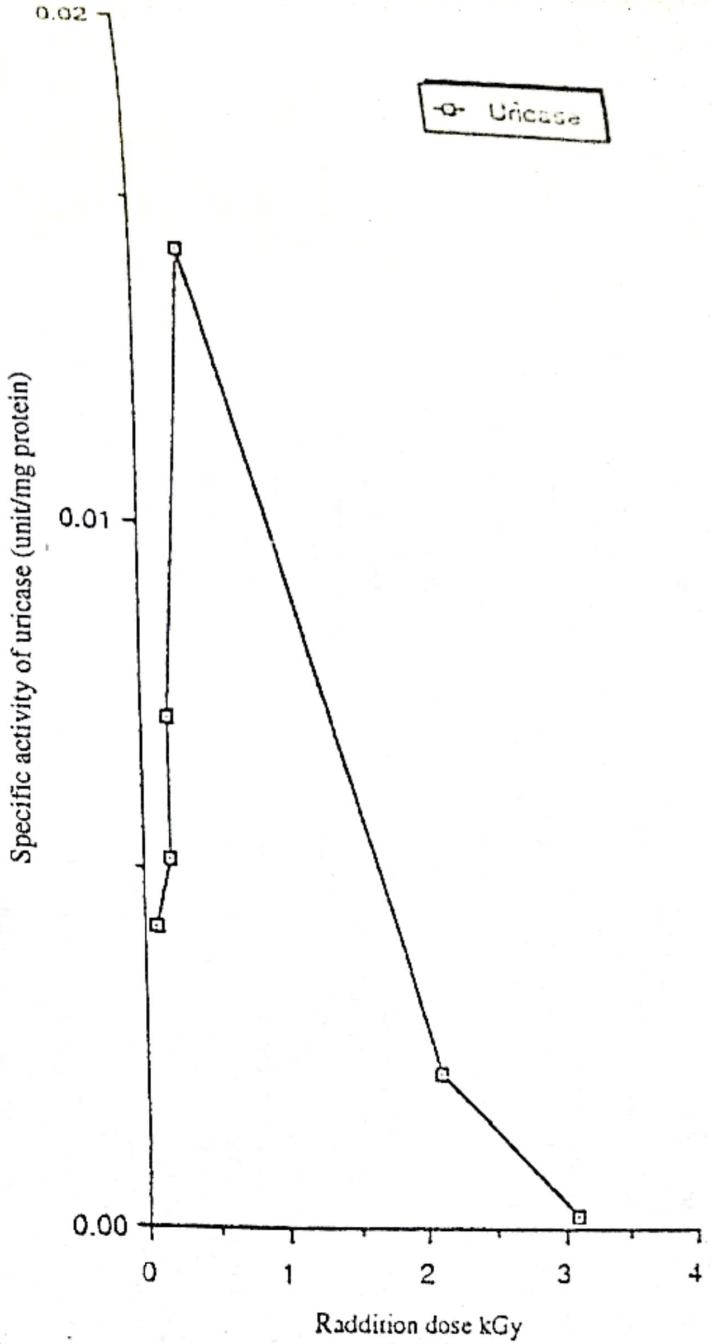

Fig. (5): Effect of gamma radiation on the purified uricase enzyme produced by *Strep. alboniger.*

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تنقية انزيم اليوريكيز من العزلة استربتوميسيتس ألبونيجر المعزولة من علف الدواجن المصرى

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قسم النبات – كلية العلوم – جامعة الزقازيق- * المركز القومى لتكنولوجيا الاشعاع بالقاهرة

تم عزل وتنقية انيزم اليوريكاز من العزلة استربتوميستيس البونيجر باستخدام سيفادكس G-200 ، وقد تم تعيين النشاط الانزيمي فى حالته النقية وكانت ٢,٢ وحدة لكل مليجرام بروتين وهى تعادل ٥٥٠ ضعف.

تم التأكد من تنقية الانزيم باستخدام أجاوز التحليل الأيونى الكهربى كما تم تعيين الوزن الجزيئى للانزيم ويقدر ب ١٣٠,٠٠٠ دالتون باستخدام البولى اكرلميد.

أظهر الانزيم المنقى أعلى نشاط عند درجة حرارة ٣٠ درجة مئوية ووسط هيروجينى رقم ٩,٠ كما تم دراسة تأثير أشعة جاما على نشاط الانزيم المنقى ووجد أن هناك نشاطا ملموسا عند جرعة اشعاعية ٠,٥ كيلو جرام بينما يحدث تثبيطا نهائيا للانزيم عند جرعة اشعاعية ٣ كيلو جرام.