Proceeding, 2nd International Conference on Radiation Sciences and Applications

28/3 – 1/4/2010 – Marsa Alam, Egypt

Characteristics of Exo-Polygalacturonase Produced by Irradiated *Aspergillus niger* and *Trichoderma viride* Spores

Youssef, Bothaina M.*; Swailam, H. M.*; EL-Mehallawy, A.A. and Gomaa, Nermein M.***

** Radiation Microbiology Department, National Center for Radiation Research and Technology(NCRRT),Atomic Energy Authority (AEA),Egypt.*

*** Microbiology department ,Faculty of Science, Ain-shams University.*

ABSTRACT

Out of 69 fungal isolates from nine pectin rich fresh fruit wastes-showed pectinolytic activity ,two were the powerful and best. They were identified as ***Aspergillus niger*** and ***Trichoderma viride***. Gamma irradiation of the spore suspensions of these two isolates (0-3kGy) stimulated the exo-polygalacturonase (PG) production. Treatment with 0.25kGy was found to be the best dose for inducing PG activity produced from ***T. viride*** and ***Asp. niger***. The enzyme characteristics were also studied. The optimum temperature of ***T. viride*** enzyme reaction was 50°C compared with 45°C for ***Asp. niger*** enzyme extract.The optimum incubation time of ***T. viride*** enzyme reaction was 80 min which greater than that of ***Asp. niger*** namely 60 min.The results of enzyme reaction pH revealed that the best PG activity was observed at pH 5.0 for the extract of the two fungal isolates. The stability of the enzyme was affected markedly by each of incubation temp., incubation period and pH value. ***Aspergillus niger*** and ***T. viride*** crude extract enzymes were stimulated with Mn^{2+} while Zn^{2+} and Ca^{2+} were inhibitors. The best volume of crude enzyme extract was 3.00 ml in case of ***T. viride*** while in case of ***A. niger*** was 2.00 ml . ***Trichoderma viride*** enzyme extract showed its highest enzyme activity with substrate concentration 1.5 % while that of ***A. niger*** was found to be 3.0%.

INTRODUCTION

Pectinolytic enzymes or pectinases are a heterogeneous group of related enzymes that hydrolyze the pectic substances [(1)]. Pectinolytic enzymes are widely distributed in higher plants and microorganisms. Most commercial pectinase preparations used in the food industry are derived from *Aspergillus niger,* which in addition to producing large quantities of these enzymes is a GRAS (Generally Recognized as Safe) microorganism [(2)].

Pectinases have been used in several conventional industrial processes, such as textile, plant fiber processing, tea and coffee fermentation, oil extraction, treatment of industrial wastewater, containing pectinacious material, etc.They have also been reported to work on purification of viruses and in making of paper [(3&4)]. Enzymes are protein molecules. Extreme conditions such as high temperature or extreme pH will affect the structure of an enzyme. This will in turn affect the catalytic activity [(5)].

The use of mutagenic factors in the improvement of the enzyme production properties of microorganisms is well known [(6-9)]. One of the main mutagenic factors is radiation. However, inductions of mutation followed by selection have proved to be the most efficient programs for the isolation of highly productive cultures [(10)].

In a previous study by the author [11],the optimum nutritional and environmental parameters for fungal pectinase production by 12 isolates from 8 pectin-rich wastes were assesd .Two of which were the powerful; they identified as ***Aspergillus niger*** **and** ***Trichoderma viride*** **.**In the present work,the effect of different γ-radiation doses on the growth and exo- polygalacturonase production by the isolated ***T. viride*** and ***Asp. niger*** was done. Extended study on the characteristics of the crude enzyme extract was also assessed.

MATERIALS AND METHODS

Preparation of the fungal spore suspensions:

This step was done to prepare the inoculum for the fermentation process. A suitable number of 250 ml Erlenmyer-flasks with 100 ml potato dextrose agar (PDA) were inoculated separately with a fungal disc of ***T. viride*** and ***Asp. niger***. These discs were taken with a cork porer from the pure plates.The PDA flasks were incubated at 28°C for 5 days. After the completion of incubation process, 10 ml of sterile solution containing (0.1% Tween 80) were added to each flask and the spores were scratched by sterile needle. Then the spore suspension was centrifuged twice at 3000 rpm for 15 min. The suspended spores for each isolate were collected and adjusted to 4x 10^6 cfu/ml by sterile saline solution.

Extract of crude Exo- Polygalacturonase;

The basic mineral medium (BMM, 100 ml) according to [12] supplemented with 1% commercial pectin was mixed in 250 ml Erlynmyer-flasks. The flasks were autoclaved at 121˚ C for 15 min and inoculated with 0.5 ml spore suspension (4x10^6 cfu/ml) of the fungal isolates ***T. viride*** or ***Asp. niger*** which isolated from pectin rich wastes. The inoculated flasks were incubated at 28˚ C as stationary cultures for 5 days. Duplicate flasks were taken daily, filtered through Whatman paper No. 3 and centrifuged under cooling conditions. The filtrate was used as the crude enzyme preparation.

Effect of gamma irradiation:

Irradiation process was carried out at the National Center for Radiation Research and Technology (NCRRT). The irradiation facility used was the Cobalt-60 (^{60}Co Indian Facility) with a dose rate 0.1 kGy/min. at the time of experiments.

To study the effect of different doses of gamma radiation on fungal growth polygalacturonase production, 3ml of the spore suspension (4x10^6 cfu/ml) as an inoculum of each microorganism separately were put in sterilized tubes under aseptic conditions. The spore suspensions were irradiated at 0.25, 0.5, 0.75, 1.0, 1.5 and 2.0 kGy at the ambient temperature. The flasks with 100 ml BMM inoculated with 0.5 ml of the irradiated spore suspension. The flasks were incubated at the suitable temperature and for the specific time after inoculation period, the fermented media were filtered through filter paper Whatman No. 3, and the supernatants were used to estimate reducing sugar (RS) and enzyme activity (EA) for each sample. The collected growth was washed three to four times with distilled water and then dried at 60˚C to a constant weight(dry biomass). All samples were carried out in triplicates.

Enzyme activity determination :

The enzyme activity was assayed as reported by [12]. Exopectinolytic activity in cell - free filtrates was assayed by quantification of reducing sugars that were liberated by 0.1ml filtrate mixed

with 0.5ml 1.0% pectin and 0.4ml acetate buffer pH 5.0 incubated for 20 minutes at 45˚C. Results were expressed as galacturonic acid equivalents. One unit (U) of exopectinolytic activity was defined as the amount of enzyme that catalyzes the formation of 1µ mol galacturonic acid under the assay conditions. Reducing groups in culture media were determined directly in cell - free filtrates using the dinitrosalicylic acid (DNS) method [13].

Reducing sugar determination:

The reducing sugar concentration was determined using DNS reagent [13]. Reducing sugars measurement was an indicator for the exo-type polygalacturonase.

Determination of Enzyme characteristics:

The features and characteristics of the crude enzyme extract were assayed by determining the following parameters:

A. Effect of incubation temperature on the enzyme activity:

Crude enzyme extract was distributed into equal aliquots to be incubated at different temperatures (20, 30, 40, 45, 50 and 60°C). After each incubation for 20 minutes, the enzyme activity was measured by the method described previously by [12].

B. Effect of incubation time on the enzyme reaction:

Crude enzyme extract was distributed into equal aliquots to be incubated at different time periods (10, 20, 30, 40, 50,60, 70, 80, 90, 100 and 120 minutes) at suitable temperature. The enzyme activity was then determined for each treatment.

C. Effect of different pH values on the enzyme activity:

Enzyme extract was divided into equal samples. Each sample was incubated at different pH values to determine the effect of the pH of the buffer on the enzyme activity. The pH values were: (3, 3.5, 4.0, 4.5, 5.0, 5.5, 6.0, 6.5, 7.0, 7.5, 8.0, 8.5 and 9.0). The enzyme activity was determined for each treatment.

D. **pH stability of the enzyme:**

Enzyme extract was divided into equal samples. Samples were incubated at different pH values with different incubation time periods. The pH range was (3, 4.0, 5.0, 6.0, 7.0, 8.0 and 9.0) and incubation periods were (60 and 80 min). Enzyme activity was finally measured for each sample.

E. Thermal stability of the enzyme:

Enzyme extract was divided into equal samples, and were incubated at different temperature degrees (10, 20, 30, 40, 50, 60, 70 and 80°**C**) with different incubation periods (60 and 80 min). Enzyme activity was finally measured for each sample.

F. Effect of metal ions on the enzyme activity:

Known volumes of the culture filtrates were divided into equal volumes to which the metal ions were added. Each salt was added in two concentrations 1mM &5mM, separately.The metal ions were $ZnSO_4$, $CuSO_4$, $FeSO_4$, $MnSO_4$, $MgSO_4$ and $CaSO_4$. Incubating samples at appropriate temperature and also for the best time 45° C/ 60 min & 50ºC/ 80 min for ***A. niger*** and ***T. viride***

extracts, respectively. The effect of metal ions was assessed by measuring the existed polygalacturonase activity.

G. Effect of enzyme extract volume on the enzyme activity:

Different volumes of the crude enzyme extract were used to be included in the enzyme reaction. These volumes were 0.1, 0.25, 0.5, 1.0, 1.5, 2.0 & 3.0 ml. Each sample run for 60 min in case of ***A. niger*** and for 80 min in case of ***T. viride*** and at convenient temperature. Finally, the enzyme activity for each sample was determined.

H. Effect of substrate concentration on the enzyme activity:

Different concentrations of substrate (citrus pectin) were prepared. These concentrations were 0.5,1.0, 1.5, 2.0 and 3.0% w/v. Each concentration was introduced in each sample reaction and incubated for the suitable time at suitable temperature. Each enzyme activity was evaluated for each sample.

RESUITS and DISCUSSION

Effect of gamma radiationon exo-polygalacturonases production:

The effect of gamma radiation doses was carried out on the the two selected fungal isolates ***Trichoderma viride*** and ***Aspergillus niger***. This study aimed to assay if radiation is valuable and significant on the production of exo-PG from the selected fungal genera under study or not and also with which doses this is true.

Data in Table (1) revealed that exposure of spore suspension of ***T. viride*** to gamma radiation at 0.25kGy led to a higher enzyme activity and a higher production of reducing sugars (31.03 U/ml and 6.65 μM/ml, respectively) compared to the non-irradiated spore suspension or those exposed to the other doses. On the other hand, the dry weight of ***T. viride*** showed the highest value (2.46 g/100ml) using the non-irradiated spore suspension and by increasing the irradiation dose levels there was a significant decrease of the dry growth weight.

Table (1):Effect of different γ-radiation doses on exo-polygalacturonase production by *Trichoderma viride* & *A. niger* grown on BMM broth with1% citrus pectin.

Radiation dose (kGy)	*Trichoderma viride*			*Aspergillus niger*		
	Reducing sugar (μM/m)	Enzyme activity (U/ml)	Dry growth weight (g/100ml)	Reducing sugar (μM/ml)	Enzyme activity (U/ml)	Dry growth weight (g/l00ml)
0.00	5.68	26.5	2.46	10.92	54.6	5.17
0.25	6.65	31.03	0.96	12.77	63.88	4.75
0.50	4.86	22.71	0.38	12.30	61.59	3.94
0.75	4.10	19.15	0.27	10.71	50.74	3.27
1.00	3.51	16.38	0.13	9.98	46.58	1.79
1.50	3.35	15.62	0.07	8.29	38.69	0.86
2.00	2.28	10.65	0.03	5.36	25.00	0.69

Inoculum size 1.0 % spore suspension (4 x10^6 cfu/ml).

The exposure of **A. niger** spore suspension to 0.25 kGy led to higher production of both enzyme activity and amount of reducing sugars (63.88 U/ml & 12.77µM/ml respectively) Table, 1. The effect of gamma radiation on the dry weight of **A. niger** was shown in the same table; the highest dry weight (5.17 g/100ml) was also obtained from the non-irradiated spore suspension as compared with the irradiated spores.

The attained results revealed that γ-irradiation of spore suspension with 0.25 kGy was found to be a stimulator for exo-polygalacturonase production by both ***T. viride*** or ***A. niger***

.

(2) **Characterization of the PG enzyme preparations:**

Extended study on the characters of crude enzyme extracted from ***T. viride*** or ***A. niger*** was done which includes the following parameters

2-1- Effect of incubation temperature of enzyme reaction on enzyme activity:

T. viride extract showed a requirement of 50°C as the EA was (34.32 U/ml) Table(2). *A. niger* extract differed as it required an enzyme reaction temperature of 45°C with best EA (64.12 U/ml) Table(2).

Table (2): Effect of different incubation temperatures of the enzyme reaction on exo-polygalacturonase activity produced by *T. viride* & *A. niger* irradiated at 0.25 kGy.

Temperature degrees (°C)	*T. viride*		*A. niger*	
	Reducing sugars (µM/ml)	Enzyme activity (U/ml)	Reducing sugars (µM/ml)	Enzyme activity (U/ml)
20	4.80	22.47	9.494	47.471
30	5.68	26.59	10.517	52.588
40	6.21	29.09	11.66	58.3
45	6.93	32.44	12.82	64.12
50	7.32	34.32	12.568	62.862
60	5.31	24.85	10.6	50.0

Among the PGases obtained from different microbial sources, most have optimal temperature range of 30–50°C[14]. Also [15] stated that maximum EA of PG for *A. niger* MUIG 16 had an ideal temperature 40°C for the enzyme reaction. Results recorded within this study coincided with the researchers records.

2-2- Effect of incubation time of enzyme reaction on enzyme activity:

Second parameter studied was the incubation time of enzyme reaction. The best incubation time of enzyme reaction in case of *T. viride* extract was 80 min (39.40 U/ml) Table(3). However, it was also recorded that *A. niger* extract demanded 60 min for best PG activity of 71.00 U/ml . It was observed that by increasing the incubation time up to 120 min there was a significant decrease in EA.

Table(3): Effect of different incubation periods of enzyme reaction on exo-polygalacturonase activity produced by *T. viride* & *A. niger* irradiated at 0.25 kGy

Incubation time (min)	*T. viride*		*A. niger*	
	Reducing sugars concentration (µM/ml)	Enzyme activity (U/ml)	Reducing sugars concentration (µM/ml)	Enzyme activity (U/ml)
10	2.55	12.03	6.67	33.35
20	7.32	34.00	12.82	64.12
30	7.89	36.65	13.20	66.04
40	7.98	37.65	13.38	66.92
50	8.05	37.94	13.84	69.20
60	8.12	38.32	14.20	71.00
70	8.31	39.21	12.68	63.40
80	8.76	39.40	12.15	60.77
90	7.55	35.62	10.00	49.98
100	6.98	32.94	8.54	42.72
120	6.20	29.71	6.84	34.19

Similarly, *A. niger* CECT 2088 had its maximum endopolygalacturonase activity with 1 h reaction time at 46°C and pH 4.8 [16].

2-3- Effect of pH of reaction medium on Enzyme activity:

Results for the effect of pH on the enzyme reaction are presented in Table(4). Exract from ***T. viride*** showed best enzyme activity at pH 5.0 with EA(39.40 U/ml). By increasing the pH value from 3.0-5.0, EAs elevated gradually.While, from pH5.5 the EAs decreased being almost non-measurable within pH 7-9. ***A. niger*** showed best enzyme activity at pH 5.0 with EA 71.00 U/ml (Table 4). That was in accordance with [15] as they reported that ***A. niger*** MUIG 16 needed pH 4.5 as the optimum enzyme reaction pH for best PG production. Also two endo-PGases (PG I and PG II), isolated from ***Aspergillus niger*** have optimal pH range of 3.8–4.3 and 3.0–4.6, respectively [14].

Table(4): Effect of different pH values of enzyme reaction on exo-polygalacturonase activity produced by *T. viride* & *A. niger* irradiated at 0.25 kGy.

Reaction pH	*T. viride*		*A. niger*	
	Reducing sugars concentration (µM/ml)	Enzyme activity (U/ml)	Reducing sugars concentration (µM/ml)	Enzyme activity (U/ml)
3.0	3.91	19.57	8.42	42.14
3.5	4.55	22.77	8.83	44.39
4.0	5.02	25.10	10.31	51.53
4.5	6.41	32.03	13.29	66.44
5.0	8.76	39.40	14.20	71.00
5.5	5.95	29.75	9.54	47.68
6.0	2.71	17.23	8.04	40.20
6.5	1.10	5.50	2.46	12.29
7.0	0.61	3.07	1.04	5.22
8.0	0.17	0.88	0.58	2.92
9.0	0.06	0.28	0.11	0.53

2-4- pH stability of the enzyme activity:

The enzyme activity of ***T. viride*** extract showed its best value of 39.40 U/ml at pH 5.0 after 80 min of incubation (Table 5). The enzyme activity kept about 43.74% of its value when the pH was increased to 6.0 as EA was (17.23 U/ml). By increasing the pH value to 9.0 the enzyme activity was lost by about 99% compared to the activity at pH 5.0. In case of ***A. niger***, the best enzyme activity was at pH 5.0 (64.33 U/ml & 49.98 U/ml) after 60 and 80 min, respectively (Table 6). The activity decreased by 43.4 % at pH 6.0 after 60 min. The decrease continued till the alkaline pH 9.0 as the activity lost by 99.2 % after 60 min. In the acidic pH, ***T. viride*** extract lost about 50 & 36.3 % of the activity at pH 3.0 & 4.0, respectively after 80 min. While, ***A. niger*** extract lost about 40.5 & 27.5 % of the activity at pH 3.0 & 4.0, respectively after 60 min. That was in agreement with results found by [17] observed that the PGase produced by

Trichoderma harzianum was very stable at pH 5.0 and retained 49% and75% of its activity at pH 4.0 and 6.0, respectively. The enzyme lost about 70–80% of its activity at pH 3.0 and pH's from 7.0 to 11. The same optimum pH was reported for PGs from ***Asergillus niger*** [18]**.**

Table(5): Effect of different pH values of enzyme reaction on exo-polygalacturonase activity produced by *T. viride* irradiated at 0.25 kGy after 60& 80 min.

Reaction pH	Reducing sugars concentration (μM/ml)		Enzyme activity (U/ml)		Recovery of enzyme activity %	
	60 Min	80 Min	60 min	80 min	60 min	80 min
3.0	3.70	4.20	17.24	19.57	44.90	49.70
4.0	2.81	5.38	13.12	25.10	34.00	63.70
5.0	8.22	8.45	38.32	39.40	100.00	100.00
6.0	2.76	3.69	12.89	17.23	33.60	43.70
7.0	0.54	0.66	2.53	3.075	6.60	7.80
8.0	0.18	0.19	0.85	0.88	2.20	2.20
9.0	0.13	0.06	0.63	0.28	0.64	0.70

Table(6): Effect of different pH values of enzyme reaction on exo-polygalacturonase activity produced by *A. niger* irradiated at 0.25 kGy after 60& 80 min.

Reaction pH	Reducing sugars concentration (μM/ml)		Enzyme activity (U/ml)		Recovery of enzyme activity %	
	60 Min	80 min	60 min	80 min	60 Min	80 min
3.0	8.22	7.29	38.34	34.01	59.50	68.00
4.0	10.01	7.57	46.69	35.32	72.50	70.60
5.0	13.79	10.72	64.33	49.98	100.00	100.00
6.0	7.81	6.80	36.42	31.74	56.60	63.50
7.0	1.12	3.79	5.22	17.66	8.00	35.00
8.0	0.63	2.99	2.92	13.97	4.50	27.90
9.0	0.11	2.64	0.53	12.34	0.82	24.70

2-5- Thermal stability of the enzyme activity:

The enzyme activity of ***T. viride*** extract was at its tenzyme activity lost by about 15 %, 28 % & 39 % at 60, 70 & 80°C, respectively. The enzyme activity of ***A. niger*** also decreased when compared with the ideal reaction temperature. The best reaction temperature was 45°C after 60 min of incubation as the enzyme activity was 71.00 U/ml Table(8). The enzyme activity decreased by 14.4 %, 37.6 %, 46.1 % and 59.4 % at 50, 60, 70 and 80°C. Similar results were recorded with ***T. reesei*** PG1 and PG2 which were stable up to 40 and 50◦C, respectively [19]**.** For ***A. niger*** PG, the optimum activity occurred at 40°C. From 30° to 40° C the rate exponentially increases. At about 50°C the activity drops rapidly [20]**.**

Table (7): Effect of different temperature degrees of enzyme reaction on exo-polygalacturonase activity produced by *T. viride* irradiated at 0.25 kGy after 60& 80 min.

Reaction temperature (°C)	Reducing sugars concentration (µM/ml)		Enzyme activity (U/ml)		Recovery of enzyme activity %	
	60 Min	80 Min	60 min	80 Min	60 min	80 min
30	6.19	5.84	29.23	27.57	76.20	69.90
40	6.93	5.57	32.71	26.29	85.40	66.70
45	7.65	8.02	36.08	37.41	94.20	94.90
50	8.12	8.76	38.32	39.40	100.00	100.00
60	5.92	7.14	27.92	33.66	72.80	85.40
70	3.84	5.71	18.10	26.95	47.20	68.40
80	2.84	4.91	13.40	23.19	34.90	58.80

Table (8): Effect of different temperature degrees of enzyme eaction on exo-polygalacturonase activity produced by *A. niger* irradiated at 0.25 kGy after 60& 80 min.

Reaction temperature (°C)	Reducing sugars concentration (µM/ml)		Enzyme activity (U/ml)		Recovery of enzyme activity %	
	60 min	80 min	60 min	80 Min	60 min	80 min
30	11.85	7.53	59.28	35.27	83.50	70.50
40	12.35	10.11	61.75	47.32	86.90	94.60
45	14.20	10.68	71.00	49.99	100.00	100.00
50	12.16	10.00	60.79	46.81	85.60	93.60
60	8.87	6.88	44.34	32.20	62.40	64.50
70	7.66	5.28	38.29	24.73	53.90	49.50
80	5.77	4.19	28.83	19.60	40.60	39.20

2-6- Effect of metal ions on the enzyme activity:

It was found in the current study that Mn^{2+} was stimulator for ***T. viride*** in both concentrations 1 & 5 mM as the enzyme activity was 43.50 & 41.12 U/ml Table(9). It is clear that the concentration 5mM of Mn^{2+} was more stimulating than 1mM. All other ions were depressors and inhibitors when compared with the sample without any treatment of ions. From Table 10, it clear that ***A. niger*** crude extract enzyme showed an affinity towards Mn^{2+} at concentration 1mM & 5mM, as the enzyme activities were 75.84 and 73.15 U/ml, respectively. Also, from Table(10) it was noticed that the other

metal ions at conc. 1 & 5 mM, lowered greatly the enzyme activity of ***A. niger***. The effect of different metal cations at the concentration of 1mM on ***T. harzianum*** PGII assay system revealed that all the examined metal cations showed different and partial inhibitory effects on the activity of enzyme except for Mn^{2+} and Co^{2+} were completely inhibited the polygalacturonase activity [17]**.** The activity of ***Sporotichum thermophile*** Apinis PG was stimulated by Fe^{2+} and Mn^{2+} at both 1 and 5 mM, while Ca^{2+} and Cu^{2+} stimulated only at 1 mM, and inhibited at 5 mM. Mg^{2+} strongly inhibited enzyme activity [21].

Table (9): Effect of metal ions with 1mM & 5mM concentration included in the enzyme reaction on exo-polygalacturonase activity produced by *T.viride* irradiated at 0.25 kGy

Metal ion as sulfate salt	1mM concentration		5mM concentration	
	Reducing sugars concentration (µM/ml)	Enzyme activity (U/ml)	Reducing sugars concentration (µM/ml)	Enzyme activity (U/ml)
Control	8.45	39.40	8.45	39.40
Zn^{2+}	7.55	35.23	7.94	37.05
Cu^{2+}	7.39	34.48	7.73	36.07
Fe^{2+}	6.99	32.61	7.96	37.11
Mn^{2+}	9.33	43.50	8.82	41.12
Mg^{2+}	7.76	36.21	7.35	34.30
Ca^{2+}	7.75	36.13	7.46	34.80

metal ions in the form of sulfate salt

Table (10): Effect of metal ions with 1mM & 5mM concentration included in the enzyme reaction on exo-polygalacturonase activity produced by *A. niger* irradiated at 0.25 kGy

Metal ion as sulfate salt	1mM concentration		5mM concentration	
	Reducing sugars concentration (µM/ml)	Enzyme activity (U/ml)	Reducing sugars concentration (µM/ml)	Enzyme activity (U/ml)
Control	15.22	71.00	15.22	71.00
Zn^{2+}	14.45	67.39	15.12	70.53
Cu^{2+}	13.48	62.88	15.31	71.39
Fe^{2+}	14.13	65.91	14.82	69.13
Mn^{2+}	16.26	75.84	15.68	73.15
Mg^{2+}	8.54	39.82	14.58	68.02
Ca^{2+}	15.02	70.05	15.54	72.50

metal ions in the form of sulfate sal

2-7- Effect of enzyme extract volume on the enzyme activity:

The best volume of crude enzyme extract was 3.00 ml in case of ***T. viride*** as the enzyme activity was 49.36 U/ml (Table, 11). The lowest enzyme activity was recorded when 0.1 ml of crude extract was used in the enzyme reaction. It was noticed that the enzyme activity and the reducing sugar concentration was increased by increasing the volume of the enzyme within the reaction mixture. The best enzyme volume in case of ***A. niger*** included in the enzyme reaction was 2.00 ml with enzyme

activity 84.85 U/ml (Table,11). It was noticed that the enzyme volumes of 1.00 ml, 1.50 ml and 2.00ml were close and approximately had the same effect on the enzyme activity as the enzyme activities were 84.10, 84.37 and 84.85 U/ml respectively. By increasing the enzyme volume, the enzyme activity increased. The enzyme activity decreased with 3.00 ml of enzyme activity as it was 83.61 U/ml.

Table (11): Effect of different enzyme extract volumes on exo-polygalacturonase activity produced by *T. viride*& *A. niger* irradiated at 0.25 kGy.

Enzyme extract volume (ml)	*T. viride*		*A. niger*	
	Reducing sugars concentration (µM/ml)	Enzyme activity (U/ml)	Reducing sugars concentration (µM/ml)	Enzyme activity (U/ml)
0.10	8.45	39.40	15.22	71.00
0.25	9.04	42.17	15.50	72.32
0.50	9.70	45.22	16.21	75.60
1.00	9.98	46.57	18.03	84.10
1.50	10.13	47.24	18.09	84.37
2.00	10.26	47.84	18.19	84.85
3.00	10.58	49.36	17.93	83.61

2-8- Effect of substrate concentration on the enzyme activity:

Table 12 show that ***T. viride*** enzyme extract showed its highest enzyme activity (54.52 U/ml) with substrate concentration 1.5 %. By increasing the substrate concentration from 0.5 % up to 1.5 %, the enzyme activity increased gradually. Beyond the substrate concentration 1.5 % the enzyme activity decreased but to a little extent not dramatic one. The lowest enzyme activity (44.70 U/ml) was recorded with substrate concentration of 0.5 %. In case of ***A. niger*** crude extract, by increasing the substrate concentration, the enzyme activity increased all over the covered range from 0.5 % till 3% (Table 12). The maximum enzyme activity (98.09 U/ml) was reported with substrate concentration 3% while the lowest EA (82.79 U/ml) was recorded with 0.5%.

Table(12): Effect of different substrate concentrations on exo-polygalacturonase activity produced by *T. viride* & *A. niger* irradiated at 0.25 kGy.

Substrate conc. % (w/v)	*T. viride*		*A. niger*	
	Reducing sugars concentration (µM/ml)	Enzyme activity (U/ml)	Reducing sugars concentration (µM/ml)	Enzyme activity (U/ml)
0.5	9.58	44.70	17.75	82.79
1.0	10.58	49.36	18.19	84.85
1.5	11.69	54.52	19.23	89.69
2.0	11.60	54.12	20.81	97.06
3.0	11.48	53.54	21.03	98.09

REFERENCES

(1) -Jayani, R. S.; Saxena, S. and Gupta, R. (2005): Microbial pectinolytical enzymes: a review. *Process Biochem.* 40: 2931–2944.

(2) -Pariza, M. W. and Foster, E. M. (1983): Determining the safety of enzymes used in food processing. *J. Food Prot.*, 46: 453- 458.

(3) -Reid, I. and Richard, M. (2004): Purified pectinase lowers cationic demand in peroxide-bleached mechanical pulp. *Enzyme Microbial. Technol.,* 34: 499-504.

(4) -Viikari, L.; Tenakanen, M. and Suurnakki, A. (2001): Biotechnology in the pulp and paper industry. In"Biotechnology" (Rehm, H. J., ed.) pp. 523– 546. VCH- VCH-Wiley.

(5) -Theresa, M. C. T. (2003): Enzymology. In "Microbial Biotechnology- principles and applications" (Lee, Y. K. ;ed.) pp.73-92. National University of Singapore. *World Scientific publications Co. Ltd.*

(6) -Shah, N. K.; Nehete, P. N.; Shah, V. D. and Kothari, R. M. (1989): Isolation of a stable and high yielding α-amylase mutant of *Bacillus subtilis. J. Biotechnol.,* 11: 67.

(7) -Kumakura, M. (1993): Dose-dependency of radiation on enzyme production in *Trichoderma reesei. Rad. Environment. Biophys.,* 32(1): 41.

(8) -Rajoka, M. I.; Bashir, A.; Hussain, S. R. S. and Malik, K. A. (1998): γ-Ray induced mutagensis of *Cellulomonas biazota* for improved production of cellulases. *Folia Microbiol.,* 43(1):15.

(9) -El-Batal, A. I. and Abdel-Karem, H. (2001): Phytase production and phytic acid reduction in rapeseed meal by *Aspergillus niger* during solid state fermentation. *Food Res. Int*., 34: 715.

(10) -Thoma, R. W. (1971): Use of mutagens in the improvement production strains of microorganisms. *Folia Microbiol.*, 8: 197-204.

(11) -El-Mehallawy A. A., Youssef, Bothaina M.; Swailam,H . M. and Gomaa, Nermein M.(2009):Pectinase Production by some fungi and its application *.N. Egypt.J. Microbiol. Vol.23,May,2009.*

(12)-Minjares-Carrenco, A.; Trejo-Aguilar, B. A.; Aguilar, G. and Viniegra-Gonzalez, G. (1997): Physiological comparison between pectinase-producing mutants of *Aspergillus niger* adapted either to solid-state fermentation or submerged fermentation. *Enz. Microbial Technol*., 21: 25-31.

(13) -Miller, G. L. (1959): Use of dinitrosalicylic acid reagent for determination of reducing sugars. *Anal. Chem.,* 31: 426 – 428.

(14) -Singh, S. A. and Rao, A. G. A. (2002): A simple fractionation protocol for, and a comprehensive study of the molecular properties of two major endopolygalacturonases from *Aspergillus niger*. *Biotechnol. Appl. Biochem.,* 35: 115–23.

(15) -Dinu, D.; Nechifor, M. T.; Stoian, G.; Costache, M. and Dinischiotu, A. (2007): Enzymes with new biochemical properties in the pectinolytic complex produced by *Aspergillus niger* MIUG 16. J. *Biotechnol*., 131: 128–137.

(16) -Rodríguez-Nogales, J. M.; Ortega, N.; Perez-Mateos, M. and. Busto, M. D.(2007):Experimental design and response surface modeling appliedfor the optimisation of pectin hydrolysis by enzymes from *A. niger* CECT 2088 *Food Chem.* 101: 634–642

(17) -Mohamed, S.A.; Farid, N.M.; Hossiny, E. N. and Bassuiny, R.I.(2006):Biochemical characterization of an extracellular polygalacturonase from Trichoderma harzianum. *J. Biotechnol*. 127: 54-64.

(18) -Behere, A.; Satyanarayan, V. and Padwal-Desai, S. R. (1993): Separation and limited characterization of three polygalacturonases of *Aspergillus niger. Enzyme Microbiol. Technol*., 15: 158-161.

(19) -Mohamed, S. A.; Christensen, T. M. I. E. and Mikkelsen, J. D. (2003): New polygalacturonases from *Trichoderma reesei*: Characterization and specificities to partially methylated and acetylated pectins. *Carbohydr. Res.,* 338: 515-524.

(20) -Nikolic´, M. V. and Mojovic, L. (2007): Hydrolysis of apple pectin by the coordinated activity of pectic enzymes. *Food Chem.* 101: 1-9.

(21) -Kaur, G.; Kumar, S. and Satyanarayana, T. (2004): Production, characterization and application of a thermostable polygalacturonase of a thermophilic mould Sporotrichum thermophile Apinis. *Biores. Technol.,* 94:239-243.

خصائص انزيم اكسو جلاكتويورينيزالمنتج بواسطة جراثيم فطرى اسبرجلس نيجر وترايكودرما فيردى المعاملة بالاشعاع

بثينة محمد يوسف *– هشام سويلم* - عادل المحلاوى نيرمين جمعه***

*قسم الميكروبيولوجيا الاشعاعيه بالمركز القومي لبحوث و تكنولوجيا الاشعاع- هيئة الطاقة الذرية. مصر
**قسم الميكروبيولوجي- كلية العلوم- جامعة عين شمس .

تم اختيار أفضل عزلتان فطريتان من بين 69 عزلة من حيث قدرتها على تكسير البكتين و احداث تغيير فى اللزوجة و انتاج السكريات المختزلة فى بيئة النمو . وتم تعريفها على انها سلالة من اسبرجلس نيجر (معزولة من مخلف بنجر السكر) و الاخرى ترايكودرما فيردى (معزولة من مخلف قشر الموز).

و تم معاملة معلق جراثيم كلا الفطرين بجرعات مختلفة من أشعة جاما تصل الى 3 ك جراى . وقد وجد ان المعاملة بجرعة قدرها 0.25 ك جراى لها تأثيرمحفز لانتاج انزيم اكسوجلاكتويورنيز من فطر ترايكودرما فيردى بينما تراوحت الجرعة المثلى لانتاجه من فطر اسبرجلس بين 0.25 – 0.50 ك جراى بالمقارنة بالمعلق الجرثومى غير المشع .

وبدراسة خصائص الانزيم اتضح ما يلى :-

- درجة حرارة التحضير المثلى كانت 50° م بالنسبة للانزيم المنتج بواسطة ترايكودرما فيردى بينما كانت 45° م للمستخلص الانزيمى المنتج بواسطة اسبرجلس نيجر.
- أفضل فترة للتحضين للتفاعل الانزيمى تراوحت بين 70 – 80 دقيقة ، 50 – 60 دقيقة بالنسبة للمستخلص الانزيمى لفطرى ترايكودرما فيردى ، اسبرجلس نيجرعلى التوالى .
- كان أفضل أس هيدروجينى للتفاعل الانزيمى لكل من المستخلصين عند رقم pH 5 .
- من دراسة تأثير وجود بعض الاملاح المعدنية وجد ان سلفات المنجنيز كان لها تأثير محفز لنشاط الانزيم المستخلص من كلا الفطرين بينما معظم الاملاح الاخرى كان لها تأثير مثبط .
- وجد ان أفضل حجم من المستخلص الانزيمى كان 3 مل ، 2 مل لفطرى ترايكودرما فيردى و اسبرجلس نيجر على التوالى .
- وجد أن أفضل نسبة من مادة التفاعل (بكتين الموالح) كانت 1.5 ، 3.0 % لفطرى ترايكودرما و اسبرجلس نيجر على التوالى .

و على ضوء تلك النتائج يمكن اختيارأفضل الظروف للحصول على الكفاءة المثلى لنشاط المستخلص الانزيمى لفطرى ترايكودرما فيردى و اسبرجلس نيجر