Optimization of ligninolytic enzymes production from *Aspergillus terreus* SG-777 by solid state fermentation

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Summary

The present study aimed at producing the ligninolytic enzymes extracts by growing single and co-cultures of an indigenous *Aspergillus terreus* SG-777 utilizing solid state fermentation (SSF) using lignocellulosic substrates. A further goal was to optimize the production condition of ligninolytic enzymes by selected fungal culture and lignocellulosic substrate. The production process was further improved by optimizing a number of physical parameters such as (substrate, incubation time, moisture level, inoculum size, pH, and temperature). By optimization of different parameters, the maximum specific activities of enzymes synthesized by *Aspergillus terreus* SG-777 were observed as 0.83 U/mg for manganese peroxidase (MnP), 18.03 U/mg for lignin peroxidase (LiP) and 0.91 U/mg for laccase, when using the banana stalks as substrate after 8 days incubation at pH 5.5 and 35°C temperature with 1×10^5 spore/ml ml inoculum size, 1:5 w/v moisture content, 20:1 C:N ratio (glucose and ammonium tartarate as carbon and nitrogen supplements), 1ml of 1mM $MnSO_4$ as mediator, and 1ml of 1mM $MgSO_4.7H_2O_2$.

Keywords: Ligninolytic enzymes, *Aspergillus terreus*, Solid state fermentation.

Introduction

Ligninolytic enzymes have been applied to numerous processes such as pulp delignification, oxidation of organic pollutants, biofuels cells, textile biofinishing (1 and 2). The robust non-specific and extracellular lignin-degrading enzymes, such as lignin peroxidase (LiP), laccase (Lac), and manganese peroxidase (MnP), were responsible for lignin degradation and bioremediation capabilities of white rote fungi (WRF) (3). However, problems may arise associated with the direct application of the fungi, such as difficulties in satisfying the growth requirements on a large scale, long incubation times, and adsorption of the pollutants on fungal mycelia, in contrast, *in vitro* treatment with ligninolytic enzymes produced through white rote fungi fermentation can minimize these problems (4). The white rote fungi must secrete high activities of ligninolytic and associated enzymes in the enzyme extract. To achieve this, enzyme production must be optimized (5). The ligninolytic machinery in most basidiomycetes is highly regulated by nutrients, such as nitrogen, copper, and manganese. Their production is also affected by fermentation factors, such as medium composition, nature of carbon source, concentration of carbon source, pH of fermentation broth, fermentation temperature, amount and nature of nitrogen source, as well as the presence of inducers, mediators and organic acids, such as citric, oxalic, and tartaric acids (6).

Among the various processes used for enzyme production, solid-state fermentation (SSF), which uses lignocellulosic biomass, appears promising because it has many advantages for fungal cultivations (7). The enzymes can be isolated and purified to different extents for diverse industrial applications, and the residual biomass can be utilized as animal feed (8). Since SSF operates under low moisture conditions, bacterial contamination chances were also minimized (9). Manganese peroxidases is a heme containing glycoprotein which requires hydrogen peroxide (H_2O_2) as well as Mn^{+2} ions for its activities (10). Lignin peroxidases were glycosylated, heme containing enzymes which functionally require H_2O_2 for the oxidation of lignin related aromatic structures (11). Laccase was a dimeric or tetrameric glycoprotein containing four copper atoms which were distributed in redox sites and has the advantage of not needing H_2O_2 for

substrate oxidation, which makes the enzyme to have a broader application spectrum than peroxidases (12). The aim of the study was to produce the ligninolytic enzymes extracts by growing single and co-cultures of an indigenous *Aspergillus terreus* SG-777 utilizing solid state fermentation (SSF) using lignocellulosic substrates.

Materials and Methods

Lignocellulosic agro-industrial wastes such as Banana stalks and Corn stover were obtained from some farms in Baghdad. Another substrate such as Wheat and Rice were obtained from market respectively and Bush horse which contains (Soybean, Corn, kernels and Barley) obtained from Equestrian Club in Jadriya – Baghdad. The substrates were crushed into small pieces, oven dried (50 °C), and ground to 40 mm mesh particle size and stored in air-tight plastic jars.

A pure culture of the indigenous strain *A.terreus* SG-777 which produces all extracellular ligninolytic enzymes, isolated from horse manure was used for the present study (13). Inoculums were prepared by growing the fungus in Kirk's basal salt medium (14). Hundred ml of media it was added in Erlenmeyer flasks (500 ml) .The medium was supplemented with Millipore filtered (0.22 µm) glucose 0.5%. Spores of *A.terreus* S.G-777 added to the media and incubation at 30 °C for 5 to 10 days to obtain homogenous spore suspensions of fungi (1×10^6 spores/ ml) to use as inoculum (15).

kirk´s basal medium modified by using 5 g from Bush horses consist of (Soybean, Corn, kernels and Barley) as a substrate instead of glucose, the substrate moistened by Kirk's basal salt medium. All the flasks were autoclaved and inoculated with 4ml ($1x10^5$ spore/ ml) homogenous suspension of strain *A.terreus* SG-777. The inoculated flasks were incubated at 30 °C for 10 days. Culture flasks were harvested by 100 ml of 100 mM sodium succinate buffer, the flasks were shaken (120 rpm) for 30min. The mixture was filtered through a Watman No.1 filter paper and then centrifuged at 3000 xg for 10 min. The clear supernatants were keep at 4 °C to determine the activities of LiP, MnP and lacc. (16).

Lip assay was performed by using 2.6 ml reaction mixture containing1ml buffer of pH 3,1 ml of 4 mM veratryl alcohol (3,4–dimethoxybenzylalcohol), 500 µL of the 1 mM H_2O_2, and 100 µL of the crude enzyme. A blank contained 100 µL of distilled water instead of crude enzyme. The absorbance was read after 10 min. reaction interval at 310 nm (E_{310} =9300$M^{-1}cm^{-1}$). Enzyme activity unit was defined as µM of veratraldehyde formed veratryl alcohol per min. (14).

The activity of MnP was measured by the method of Wariishi and Valli (17). The assay mixture (2.6 ml) contained 1ml of 1mM $MnSO_4$, 1 ml of 50 mM sodium malonate buffer pH 4.5 and 100 µL of the crude enzyme. Five hundred micro liters of 0.1 mM H_2O_2 was added as an oxidizing agent. A blank contained 100 µL of distilled water instead of crud enzyme. Reaction interval at 270 nm (E_{270} =11590$M^{-1}cm^{-1}$). Manganese peroxidase activity unit was defined as µM of $MnSO_4$ (catalyses the oxidation of Mn^{2+} ions to highly reactive Mn^{3+} ions formed per min).

Laccase activity in the crude extracts was measured by the method of (18) by monitoring the oxidation of guaiacol in the reaction mixture containing 1 ml of 2 mM of guaiacol in 3 ml of 10 mM sodium acetate buffer (pH 5) and 1ml of crude enzyme. A blank contained 1ml of distilled water instead of crude enzyme. The reaction mixture was incubated at 30 °C and absorbance was taken at 450 nm after 15 min. (E_{450}= 12100 M^{-1} cm^{-1}). All ligninolytic enzyme activity was expressed as IU/ml .An international unit IU (or U) is defined as the amount of enzyme, which catalyzed the transformation of 1 micromole of substrate per minute under standard conditions. This was calculated using the formula (19):

Enzyme Activity (U/ml) = (A×V) / (t×£×v) Where:
A= Absorbance at corresponding wavelength
V= Total volume of reaction mixture (ml)
v= enzyme volume (ml)
t= Incubation time (min.)
£= Corresponding Extinction coefficient (M^{-1} cm^{-1}).

The protein concentration was estimated according to Lowry method (20), to further enhance the ligninolytic enzymes production capability of fungi, different nutritional

substrate and different physical parameters (incubation time, moisture level, inoculums size, pH and temperature) were optimized (21). Different substrates such us Bush horse which contain (Soybean, Corn, kernels and Barley), rice bare, wheat, corn stover and banana stalk were used to produce ligninolytic enzymes from *A. terreus* SG-777. Five gram from each substrates prepared in five separate set of flasks (500 ml) moistened with Kirk´s basal media (14) at pH 4.5 and 1:6 (w/v) moisture. All flasks were autoclaved, and inoculated with 5ml (1×10^6 spore/ml) of homogenous suspension of *A. terreus* SG-777.The inoculated flasks were incubated at 30 °C for 10 days. After incubation period, the culture flasks were harvested by added 100 ml of 100mM sodium succinate buffer (pH 4.5); the flasks were shaken (120 rpm) in orbital shaker for 30 min. The mixture was filtered through a Whatman No.1 filter paper, and the resulting filtrates were centrifuged at 3000xg for 10 min. (16), and these supernatant were kept at 4 °C to determine the activities and Protein concentration in paragraphs (4 and 5).

Five gram from banana stalk the best substrate prepared in separate set of flasks (500 ml) moistened with Kirk´s basal media at pH 4.5 and 1:6 (w/v) moisture. All reactor flasks were autoclaved, and inoculated with $1x10^6$ spore/ml of homogenous suspension of *A. terreus* SG-777. The inoculated flasks were incubated at 30 °C for 10 days. The culture flask was harvested at different time (48, 96, 144, 192 and 240 hr.) according paragraph (3). Enzymes activities and protein concentration was determined of ligninolytic enzymes.

The substrate banana stalk moistened with different volume of Kirk´s basal media to varying moisture levels (1:1, 1:2, 1:3, 1:4, 1:5, 1:6 w/v) before inoculation. Enzymes activities and Protein concentration were determined. Five gram from banana stalk (the best substrate) prepared in separate set of flasks (500 ml) moistened with Kirk´s basal media at pH 4.5 and 1: 5 (w/v) moisture. All flasks were autoclaved, and inoculated with different coentrations (1×10^2, 1×10^3, 1×10^4, 1×10^5, 1×10^6, 1×10^7 spore/ml) of homogenous suspension of *A. terreus* SG. Enzymes activities and Protein concentration were determined. To optimize pH for maximum enzyme production, media adjusted to varying pH (3.5, 4, 4.5, 5, 5.5 and 6). Enzymes activities and Protein concentration were determined. To optimize the temperature for growth and ligninolytic production by *A. terreus* SG-777, the flasks at optimum pH were incubated at varying temperature (20, 25, 30, 35, 40 and 45 °C). Enzymes activities and Protein concentration were determined as in paragraph (4 and 5). The Statistical Analysis System (22) was used to effect of different factors in study parameters. Least significant difference –LSD test was used to significant compare between means in this study.

Results and Discussion

The results in the (Table, 1) showed that the (18.41 U/mg), MnP (0.4 U/mg) and Lacc (0.98 U/mg) was achieved in SSF using banana stalk after 6 days incubation, followed by corn stover, maximum production of LiP (14.68 U/mg), MnP (0.35 U/mg) and Lacc (0.29 U/mg). Cost-effective production of ligninolytic enzymes is a key for successful exploitation of lignocellulosic resources as a renewable energy source (23). These results were consistent with (16) the optimum activities of ligninolytic enzyme after nine days of SSF with banana stalk. Different white rote fungi (WRF) have been reported to produce maximum ligninolytic enzymes after different time periods due to genetic variation among the strains as well as in the nature and composition of the substrates used (24).

Table, 1: ligninolytic enzymes production in different substrates by *A. terreus* SG-777

Type of Substrate	Ligninolytic enzyme	Specific activity U/mg protein
Bush horse	LiP.	3.76
	MnP.	0.30
	Lacc.	0.06
Rice	LiP.	3.28
	MnP.	0.27
	Lacc.	0.01
Wheat	LiP.	3.39
	MnP.	0.28
	Lacc.	0.04
Corn Stover	LiP.	14.68
	MnP.	0.35
	Lacc.	0.29
Banana stalks	LiP.	18.41
	MnP.	0.40
	Lacc.	0.98

There were significant differences between specific activities of Lip and Lacc production at different substrate (P≤0.05) whereas MnP have no significant differences (Table, 2). After every 48 hr, the duplicate SSF flasks were harvested and culture supernatants were analyzed for ligninolytic enzymes. The results of time course study showed that maximum specific activity of MnP (0.38 U/mg), LiP (14.31 U/mg) and lacc (0.94 U/mg) was achieved in 8 days of SSF of banana stalk by *A. terreus* SG-777. As the incubation time increased, it was observed that ligninolytic enzymes production steadily increased with an increasing fermentation time but further increase in fermentation time showed a decrease in ligninolytic enzymes activities (Table, 3).

Table, 2: Effect of substrate media and enzymes production in specific activity (U/mg).

Substrate media	Ligninolytic enzyme specific activity			LSD value
	Lip.	MinP.	Lacc.	
Fodder horse	3.76 A c	0.30 B a	0.06 B b	1.094 *
Rice	3.28 A c	0.27 B a	0.01 B b	1.185 *
Wheat	3.39 A c	0.28 B a	0.04 B b	1.264 *
Corn stover	14.68 A b	0.35 B a	0.29 B b	2.781 *
Banana stalks	18.41 A a	0.40 B a	0.98 B a	3.074 *
LSD value	2.746 *	0.293 N S	0.613 *	---
* (P<0.05).				

N S=non-significant. The identical capital letters refer to non-significant differences horizontal. The identical small letters refer to non-significant differences vertical.

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Table, 3: ligninolytic enzymes production harvested in different time interval.

Incubation times	Ligninolytic enzyme	Specific activity u/ mg
48	LiP.	3.28
	MnP.	0.08
	Lacc.	0.76
96	LiP.	8.24
	MnP.	0.16
	Lacc.	1.02
144	LiP.	11.24
	MnP.	0.30
	Lacc.	1.04
192	LiP.	14.31
	MnP.	0.38
	Lacc.	0.94
240	LiP.	13.22
	MnP.	0.35
	Lacc.	0.72

Different values of Lip specific activity at incubation period for 48, 72, 96, 144, 192 and 240 hrs. revealed significant differences (P≤0.05) while the MnP and Lacc have no significant differences recorded (Table, 4).

Table, 4: Effect of Incubation times and enzymes production in specific activity (U/mg).

Incubation times	Ligninolytic enzyme specific activity			LSD value
	Lip.	MinP.	Lacc.	
48	3.28 A d	0.08 B a	0.76 B a	1.872 *
96	8.24 A c	0.16 B a	1.02 B a	1.649 *
144	11.24 A b	0.30 B a	1.04 B a	2.507 *
192	14.31 A a	0.38 B a	0.94 B a	2.169 *
240	13.22 A a	0.35 B a	0.72 B a	2.437 *
LSD value	2.066 *	0.309 NS	0.736 NS	---
* (P<0.05).				

The identical capital letters refer to non-significant differences horizontal. The identical small letters refer to non-significant differences vertical

The moisture level (1: 5 w/v) of the culture causes an enhancement of fungal growth and ligninolytic enzymes production**.** Banana stalk that was fermented at (1:5 w/v) moisture ratio gave maximum specific activity of LiP (14.2

U/mg), MnP (0.88 U/mg) and Lacc (2.67 U/mg) production after 8 days of inoculation with *A. terreus* SG-777. Increasing the moisture level from (1:3 to 1:5 w/v) causes an enhancement of fungal growth and ligninolytic enzymes production. However, an increase in moisture level of more than 1: 5 w/v caused a significant decrease in enzyme activities (Table, 5).

Table, 5: Ligninolytic enzymes production in different moisture level.

Moisture level	Ligninolytic enzyme	Specific activity u/ mg
1:1	LiP.	26.01
	MnP.	0.73
	Lacc.	1.05
1:2	LiP.	27.54
	MnP.	0.91
	Lacc.	1.13
1:3	LiP.	29.87
	MnP.	0.89
	Lacc.	0.91
1:4	LiP.	30.02
	MnP.	0.98
	Lacc.	1.30
1:5	LiP.	50.40
	MnP.	1.15
	Lacc.	3.88
1:6	LiP.	11.19
	MnP.	0.46
	Lacc.	1.06

The specific activity of ligninolytic enzymes in high and low moisture levels were inhibited, and the low secretion of activities of enzymes due to poor accessibility of nutrients and limited aeration (24 and 25). The end product and water requirement of the fungus depends on an optimum moisture level in SSF and the water holding capacity of the substrate (15). Different values of Lip and Lacc specific activity at different moisture level revealed significant differences ($P \leq 0.05$), while the MnP have no significant differences recorded (Table, 6). The maximum activities of MnP (0.35 U/mg), LiP (13.46 U/mg) and lacc (0.28 U/ml) were produced in the flasks which were inoculated by *A. terreus* SG-777 (Table, 7).

The production of enzymes increased with an increase in inoculums from 1×10^2 to 1×10^5 spore/ ml. However, a further increasing in an inoculums volume caused a decrease in enzyme production. In previous studies (8), maximum ligninolytic enzymes production from lignin containing waste rice straw was achieved when fermentation media was inoculated with 5 ml (1×10^6 spore/ml) of freshly prepared fungal spore's suspension. Lower inoculums size may not be sufficient to promote the fungal growth resulting in longer lag phase (6 and 26) whereas higher inoculum size causes faster depletion of available nutrients required for growth (24 and 27).

Table, 6: Effect of Moisture level and enzymes production in specific activity (U/mg).

Moisture level	Ligninolytic enzyme specific activity			LSD value
	Lip.	MinP.	Lacc.	
1:1	26.01 A b	0.73 B a	1.05 B b	3.177 *
1:2	27.54 A c	0.91 B a	1.13 B b	2.624 *
1:3	29.87 A b	0.89 B a	0.91 B b	3.118 *
1:4	30.02 A b	0.98 B a	1.30 B b	3.073 *
1:5	50.40 A a	1.15 B a	3.88 B a	4.215 *
1:6	11.19 A c	0.46 B a	1.06 B b	2.294 *
LSD value	5.186 *	0.691 NS	1.056 *	---

* (P<0.05).

The identical capital letters refer to non-significant differences horizontal. The identical small letters refer to non-significant differences vertical.

Table, 7: Ligninolytic enzymes production by use different size of inoculums.

Size inoculum	Ligninolytic enzyme	Specific activity u/ mg
10^2	LiP.	10.55
	MnP.	0.18
	Lacc.	0.26
10^3	LiP.	12.24
	MnP.	0.17
	Lacc.	0.25
10^4	LiP.	13.46
	MnP.	0.35
	Lacc.	0.28
10^5	LiP.	14.85
	MnP.	0.85
	Lacc.	0.66
10^6	LiP.	13.21
	MnP.	0.33
	Lacc.	0.27
10^7	LiP.	12.26
	MnP.	0.19
	Lacc.	0.21

All the Lip, MnP and Lacc specific activity recorded at (10^2, 10^3, 10^4, 10^5, 10^6 and 10^7) spore /ml revealed significant differences (P≤0.05) (Table, 8).

Table, 8: Effect of Size inoculums and enzymes production in specific activity (U/mg).

Size inoculums	Ligninolytic enzyme specific activity			LSD value
	Lip.	MinP.	Lacc.	
102	10.55 A c	0.18 B b	0.26 B b	2.355 *
103	12.24 A bc	0.17 B b	0.25 B b	2.092 *
104	13.46 A ab	0.35 B b	0.28 B b	3.268 *
105	14.85 A a	0.85 B a	0.66 B a	3.265 *
106	13.21 A ab	0.33 B b	0.27 B b	2.796 *
107	12.26 A bc	0.19 B b	0.21 B b	2.077 *
LSD value	2.093*	0.372 *	0.274 *	---
* (P<0.05).				

The identical capital letters refer to non-significant differences horizontal. The identical small letters refer to non-significant differences vertical

The maximum specific activities of LiP (17.31 U/mg), MnP (0.31 U/mg) and Lacc (0.22 U/mg) were recorded in the SSF media processed at pH 5.5 (Table, 9).

Table, 9: Ligninolytic enzymes production by use different initial pH of medium.

pH	Ligninolytic enzyme	Specific activity u/mg
3.5	LiP.	15.72
	MnP.	0.20
	Lacc.	0.19
4	LiP.	15.95
	MnP.	0.22
	Lacc.	0.23
4.5	LiP.	17.31
	MnP.	0.31
	Lacc.	0.22
5	LiP.	16.40
	MnP.	0.30
	Lacc.	0.17
5.5	LiP.	15.53
	MnP.	0.16
	Lacc.	0.82
6	LiP.	14.06
	MnP.	0.11
	Lacc.	0.18

It was noted that ligninases production steadily increased with an increase in initial medium pH from 3.0 to 5.5 and further pH increase showed a decrease in ligninolytic enzymes production. These results suggest that ligninolytic enzymes of *A.Terreus* SG-777 was produced at wide range of pH (3 - 6), this character was useful in industrial production of ligninolytic enzymes since it is not may sensitive to any changes in medium pH. Reported that the pH optimum for ligninolytic production by Ascomycets was highly dependent on chemical composition of the substrates and fermentation media. Whit rote fungi in most of the cases have shown optimum mycelia growth to produce higher activities of ligninolytic enzymes at pH 3-6 (28). Different values of Lip, and Lacc specific activity recorded at pH 3.5, 4, 4.5, 5, 5.5 and 6, revealed significant differences (P≤ 0.05) while the MnP have no significant differences recorded at these value of pH (Table, 10).

Table, 10: Effect of PH and enzymes production in specific activity (U/mg).

PH	Ligninolytic enzyme specific activity			LSD value
	Lip.	MinP.	Lacc.	
3.5	15.72 A ab	0.20 B a	0.19 B b	2.092 *
4.0	15.95 A ab	0.22 B a	0.23 B b	1.864 *
4.5	17.31 A a	0.31 B a	0.22 B b	2.705 *
5.0	16.40 A a	0.30 B a	0.17 B b	2.613 *
5.5	15.53 A ab	0.16 B a	0.82 B a	2.299 *
6.0	14.06 A b	0.11 B a	0.18 B b	2.215 **
LSD value	2.173*	0.272 NS	0.293 *	---
* (P<0.05).				

The identical capital letters refer to non-significant differences horizontal. The identical small letters refer to non-significant differences vertical.

To optimize the temperature for growth and ligninolytic enzymes production by *A. terreus* SG-777, the culture flasks at optimum pH were incubated at varying temperatures. The maximum activities of MnP (0.83 U/mg), LiP (18.03 U/mg) and lacc (0.91 U/mg) were produced when the flasks incubated at 35°C. The specific activity of ligninolytic enzymes

increased with the temperature and peaked at 35 °C. When cultivated at temperatures higher than 35 °C, the ligninolytic enzymes activities were substantially decreased (Table, 11). A variation in incubation temperature has a significant influence on synthesis of ligninolytic enzymes and their activities. The temperatures ranging from (25 to 37 °C) have been found optimum for ligninase production by different WRF (2). All the Lip and MnP specific activity recorded at 25, 30, 35, 40 and 45 °C revealed significant differences (at P≤0.05), while the Lacc have no significant differences recorded at these value of temperature degrees (Table, 12).

Table, 11: Ligninolytic enzymes production in different temperatures.

Temperatures	Ligninolytic enzyme	Specific activity u/ mg
25	LiP.	5.26
	MnP.	0.18
	Lacc.	0.87
30	LiP.	12.41
	MnP.	0.10
	Lacc.	1.14
35	LiP.	18.03
	MnP.	0.83
	Lacc.	0.91
40	LiP.	15.17
	MnP.	0.61
	Lacc.	0.83
45	LiP.	13.28
	MnP.	0.42
	Lacc.	1.13

Table, 12: Effect of Temperatures and enzymes production in specific activity (U/mg).

Temperatures	Ligninolytic enzyme specific activity			LSD value
	Lip.	MinP.	Lacc.	
25	5.26 A c	0.18 B c	0.87 B a	1.536 *
30	12.41 A b	0.10 B c	1.14 B a	2.622 *
35	18.03 A a	0.83 B a	0.91 B a	2.967 *
40	15.17 A b	0.61 B ab	0.83 B a	2.247 *
45	13.28 A b	0.42 B b	1.13 B a	2.006 *
LSD value	2.966 *	0.375 *	0.488 NS	---
* (P<0.05).				

The identical capital letters refer to non-significant differences horizontal. The identical small letters refer to non-significant differences vertical.

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الظروف المثلى لإنتاج الإنزيمات المحللة للكنين والمنتجة من العزلة المحلية *Aspergillus terreus* SG-777 باستعمال تخمرات الحالة الصلبة

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الخلاصة

إن الهدف من هذه الدراسة هو إنتاج الإنزيمات المحللة للكنين في ظروف مثلى من العزله المحلية المنتخبة *Aspergillus terreus* SG-777 باستعمال تخمرات الحالة الصلبة. درست الظروف المثلى لإنتاج الانزيمات المحللة للكنين باستعمال عدة معاييرفيزيائية مثل (الركيزة، ومدة حضانة، ومستوى الرطوبة، وحجم اللقاح، ودرجة الحموضة، ودرجة الحرارة). أظهرت النتائج أن أعلى فعالية نوعية سجلت للانزيمات المحللة للكنين والمنتجة من العزلة المحلية المنتخبة *Aspergillus terreus* SG-777 والمعزولة من روث الخيول كانت لانزيم اللكنين بيروكسيديز 18.03 وحدة/ملغم، انزيم المنغنيز بيروكسيد 0.83 وحدة/ملغم وانزيم اللاكيز 0.91 وحدة /ملغم، باستعمال سيقان الموز كركيزة ومدة حضانه 8 ايام بأس هيدروجيني 5.5 ودرجة حرارة 35 °م وبحجم لقاح 1×10^{5} وبدرجة رطوبة 5:1 وزن /حجم من وسط الترطيب والذي يحتوي على نسبة 20:1 كاربون: نتروجين (الكلوكوز والامونيوم تارتريت كمصدر كاربوني ومكملات نتروجينية) بإضافة 1مل كبريتات المنغنيز و1مل من كبريتات المغنيسيوم المائية الى وسط الترطيب.

الكلمات المفتاحية: الإنزيمات المحلله للكنين، الأسبيرجلس، تخمرات الحالة الصلبة.