J. Agric. Sci. Mansoura Univ., 27(4): 2761 - 2774, 2002

DEGRADATION OF PLANT PATHOGENIC FUNGI BY Streptomycetes

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ABSTRACT

An isolate of *Streptomyces* sp. excreted chitinase, cellulase and protease activities into the medium when grown on chitin, carboxymethylcellulose and casein , respectively, or on cell walls of the fungal pathogen *Sclerotium rolfsii* or *Rhizoctonia solni* , as sole carbon source . This isolate of *Streptomyces* sp. gave highest lytic enzymes (chitinase , cellulase , protease), when grown at28 °C. Factors involved in the production of enzymes were investigated. The lytic activities were produced optimally in the medium at pH range between 6.5-7.0 for the three enzymes . The lytic activities of culture on autoclaved mycelium of *S. rolfsii* and *R.. solani* took place at different pH values .The effect of mycelium concentration as substrate on the extent on mycelium lysis and lytic activities were also studied. The level of hydrolytic enzymes , produced by *Streptomyces* sp. depended on the source of mycelium attacked i. e. *S. rolfsii* or *R. solani* in soil.

Keywords: *Streptomyces* sp. , Biological control , Lytic enzyme , Wilt disease.

INTRODUCTION

The cell walls of *Sclerotium rolfsii* and *Rhizoctonia solani* are composed of β –1,3-glucan and chitin (Chet *et al.* 1967 ; Bartnicki-Garcia1973),whereas a number of the *Oomycetes* .e.g. , *phythium* sp , also contain cellulose (Bartnicki-Garcia1973). Some studies have shown that the fungus *Trichoderma harzianum* and *S. relfsii* utilize cell walls as a sole carbon source (Hadar *et al*, 1979 and Hamed *et al.*, 1998) . The antagonistic ability of some organisms such as *T. harzianum, T. viridi*, *Streptomyces* sp. , *Bacillus* sp . and *Micococcus* sp. against several soil borne plant pathogens were reported by several authors (Dennis and Webster 1971 ; Saad and Nawar 2001; Abd El.Fattah 1995 and Hamed 1996). Application of the biocontrol agent to fields infested with *R. solani* and *S. rolfsii* successfully reduced disease incidence (Elad and Henis 1981a, 1981b and Elad *et al.* 1981). These cultures produced extracellular β –1,3-glucanase and chitinase when grown on cell wall of *R. solani* (Chet *et al.* 1979 ; Hadar *et al.* 1979 and Elad *et al.* 1982). It appears that the main mechanism involved in the antagonism of *T. harzianum* against the pathogenic fungi is the release of lytic enzymes that destroy the integrity of the cell walls of the pathogens.

The present work deals with the enzymatic basis of the interaction between a culture of *Streptomyces* sp. isolated from Egyptian soils and *S. rolfsii* and R. *solani* in culture and soil .

MATERIALS AND METHODS

1- Antagonistic Streptomyces :

Streptomyces strain was isolated from Egyptian soil and identified according to Bergey's Manual (1974) and was characterized as an active fungal cell wall lytic organism.

2- Pathogens :

Highly virulent strains of pathogenic fungi were previously isolated from diseased sesame and tomato plants . They were identified as *Rhizoctonia solani* and *Sclerotium rolfsii* (causing root rot disease to the same hosts) . The fungi were maintained on potato dextrose agar (PDA) slants at 5-10 °C and subcultured on fresh medium every 6-8 weeks .

Growth medium and substances :-

Synthetic medium (SM) was prepared according to Okon *et al.* (1973) containing (gm / L):

Glucose (10.0) , $MgSO_4.7H_2O$ (0.2); K_2HPO_4 (0.9) ; KCl (0.2) ; NH_4NO_3(1.0);$FeSO_4$ .7 H_2O (0.003) ; MnCl $_2$(0.02) and $ZnSO_4$.$7H_2O$ (0.002) in distilled water and the pH value was adjusted at 6.3 . The medium was used throughout this work after the addition of one of the indicated carbon source , instead of glucose. The medium was supplemented with one of the following as carbon source : chitin , N- acetylglucosamine, lactose , sucrose, cellobiose , peptone , fungal cell wall , soluble casein .

Growth of *Streptomyces* sp. in liquid culture

The *Streptomyces* sp. isolate was grown in Erlenmeyer flasks (250 ml) containing 50 ml of SM medium with the adequate carbon source on a rotary shaker at 150 rpm and incubating at 28 ± 2 °C. The 2×10^6 spores /ml, inoculated flasks were kept at 28± 2°C on a rotary shaker at 150 rpm .The level of the enzymes were estimated during 7 days of incubation. After apropriate time, cells were harvested by centrifugation at 4000 rpm and the clear supernatant was used as enzyme preparation.

The cell walls of the tested pathogenic fungi (*S. rolfsii* and *R. solani*) were prepared according to Chet *et al.* (1967) .

Effect of nutritional and environmental conditions on the lysis of fungal mycelium :

Effect of different concentrations of dead mycelium (5%,10%,15%and 20%), and effect of initial pH (5.0 –9.0) of medium on lysis of mycelium was estimated as a weight of residual mycelium after lysis at the end of the experimental period. The results were expressed as percent lysis as compared to the original wieght of the mycelium.

Dry weight of mycelium was determined after incubating at 60°C for 1 week.

Enzyme activity in soil

Loamy sand soil (Hadar *et al.* 1979) was washed twice in tap water for 1 hr. Dried samples of 30g each containing 15% moisture were autoclaved and incubated with mycelium discs of tested pathogens. After 3-4 days of incubation the soil was infested with 1ml of spore suspension of *Streptomyces sp.*(3×10^6 spores/g soil) and icubated at 28°C. (Elad and Henis 1981b). Three days later , 10 ml of M citrate phosphate buffer (pH6.5) was added to the culture . The soil was shaken for20 min. (180 rpm) and centrifuged at 4000 rpm for 10 min. The supernatant was examined for enzymatic activity.

Enzyme assay

Chitinase (EC 3.2.1.14) was assayed by following the release of N-acetyl glucosamine according to Jeuniaux (1966). One ml of the enzyme was incubated with 1 ml (1 %) colloidal chitin in 0.1 M citrate phosphate buffer (PH 6.5). The reaction mixture was incubated at 37°C for 2hr. One unit of enzyme activity is defined as the amount of enzyme required to produce 0.5µ mole per ml of N-acetylglucosamine per hour . Specific activity was expressed as units/milligram of protein per hour.

Cellulase (EC 3.2.1.4) was assayed by following the release of free glucose from carboxymethyl cellulose as a substrate . The activity was defined and measured according to Mandels *et al.* (1976).
One unit was defined as the amount of enzyme that releases 1 µ mol glucose / min.. Glucose equivalents (reducing sugars) generated during assay were estimated by using the 3, 5 dinitrosalicylic acid (DNS) method (Miller GL. (1959), with glucose as standard.

Protease (EC 3.4.24.4) activity was followed using a reactin mixture containing 1 ml of 0.05 M citrate phosphate buffer (pH 6.5) , 1 % casein and 1ml of crude enzyme.

This suspension was incubated for 1 hr. at 37°C . The reaction was stopped by adding 10 % trichloroacetic acid(TCA) , kept for another 20 min at the same temperature and followed by centrifugation at 4000 r p m. for 20 min.Samples of 75 µl were removed and tyrosine was determined according to Greenberg (1959), using Folin reagent (Folin and Ciocalteu , 1927) . One unit of enzyme is defined as the amount of enzyme which yields a colour equivalent to µ mol of tyrosine per min in 1ml of digestion mixture under the standard conditions of pH and temperature.

Partial purification of the enzymes :-
Ammonium sulphate preciptation

The culture supernatant containing the crude enzymes (chitinase , cellulase , protease) was adjusted to 30 % , 60 % and 90 % saturation with ammonium sulphate, with gentle stirring at 4°C for 30 min . To each 50 ml of crude extract , ammonium sulphate crystals were added to the extract , 8.8 g (30 %). The mixture was stirred for 10 min at 4°C . The precipitate formed was collected by cooling centrifuge at 12,000 rpm for 20 min. An additional

9.9 g (60 %) of ammonium sulphate/ 50 ml for 20 min. An additional 9.9 g (60 %) of ammonium sulphate/ 50 ml of supernatant solution was added , and the solution was centrifuged at 12,000 r.p.m for 20 min .The precipitate was then dissolved in 15 ml of 0.1 M phosphate buffer pH 6.5.

Thermal and pH stability:

Partially purified enzymes (chitinase, cellulase, protease) were tested for enzyme stability during 1hr. of incubation at a temperature range of 0-100°C and a pH range2.5-10 for 24 hr. at 23°C .The pH stability was studied using buffers (0.1M) used were either citrate phosphate (pH 2.5-6.0) , phosphate buffer (pH 6.5-8.0) or carbonate bicarbonate (pH 8.5-10.0).

RESULTS AND DISCUSSION

Some cultural conditions affecting lytic enzymes production:

- Effect of incubation period on lytic enzyme production:

Samples were taken daily and the enzyme activities were measured, during 7 days of incubation Results shown in table (1) clearly show that *Streptomyces* sp. produced variable levels of lytic enzymes along the fermentation period allowed. The pattern production of these enzymes seem to be different depending on the type of the enzyme followed . Thus the maximum levels of chitinase , cellulase and protease were reached after 4 ;5 and 2 days of incubation respectively.

Table (1): Effect of individual substrates on used inducers of lytic enzymes production by *Streptomyces* sp. through 7 days of incubation period at 28 °C

Inducers substrate at 1%	Lytic enzymes	Enzyme activity(units/mg protein)/days					
		2	3	4	5	6	7
Chitin	**Chitinase**	40	990	1700	1210	820	700
CMC	**Cellulase**	5.6	12.6	18.3	23.0	21.8	14.0
peptone	**Protease**	8.4	20.3	13.3	10.0	5.5	4.0

-Effect of initial pH on the production of lytic enzymes :-

Medium containing (chitin or carboxymethylcellulose or casein) was adjusted to different pH values (from 2.5 to 9.0) using a series of buffers namely citrate phosphate ,phosphate and carbonate bicarbonate buffers. The 250 ml conical flasks contain 50 ml medium of different pH values were inoculated with standard inocula and incubated on the shaker at 28°C . Periodical determination of the enzymes levels were carried out to determine the optimal pH for enzymes production . Data in table (2) show that maximum lytic enzymes production (chitinase ; cellulase ; protease) were obtained using media with initial pH adjusted to values around neutrality between 6.5 - 7.0 for the three enzymes . Other tested pH values resulted in considerable reduction in lytic enzymes production.

Table (2): Effect of initial pH of the medium on lytic enzymes production by *Streptomyces sp.*

Lytic enzymes	Enzyme activity (units/mg protein) at different pHs of growth medium								
	2.5	3.5	4.5	5.5	6.5	7.0	7.5	8.5	9.0
Chitinase	54.0	390	920	1430	1750	1713	1000	920	810
Cellulase	3.9	6.8	13.2	18.0	23.1	22.7	19.8	15.2	11.1
Protease	5.3	9.5	11.1	16.8	21.0	20.4	18.3	16.2	10.5

Medium was adjusted to different pHs using a series of buffers namely citrate phosphate ,phosphate and carbonate bicarbonate buffers.

- **Effect of various carbon sources on lytic enzymes production :-**

Glucose in medium was replaced by other carbon sources i.e. sucrose, chitin, cellobiose, N- acetylglucosamine, carboxymethylcellulose) or lactose at a rate of 10 g/L . Aliquots of 50 ml of the medium were poured in 250 ml conical flasks. Replicates of each treatment were inoculated with standard inocula ($2x10^6$ spores /ml) and incubated at 28°C as shake culture Treatments were quantitatively analyzed. The data obtained on the effect of various carbon sources on the appearance of lytic enzymes in the cell free culture supernatant of *Streptomyces sp.* are shown in table (3) .With the respect to chitinase activity the highest enzyme level was obtained upon growing the organism on the chitin, which is the natural substrate of this enzyme. Only traces of the enzyme activity was detected upon using N-acetylglucosamin (N.A.G.A.) or glucose. On the other hand the use of the disaccharide's lactose, sucrose or cellobiose containing glycoside linkage similar to chitin (β-1,4) did not yield chitinase into the growth medium . The results suggest that the chitinase enzyme is of inducible nature and only induced by chitin. With respect to cellulase activity the tabulated results indicate that the addition of carboxymethylcellulose in the medium as a carbon source gave the highest level of enzyme suggesting that carboxymethylcellulose acted as the best inducer of cellulase production.Low levels of cellulase activity was obtained upon using cellobiose and glucose .Other tested carbon sources failed to support cellulase production . Our results confirmed the findings of Reese and Levinson (1952), Mandels and Reese (1957), in *Trichoderma viridi*, Kassim (1983) in *Aspergillus niger* and Elad *et al.* (1982) in *Trichoderma harzianum.* In the present work small amount of the enzyme were produced in the presence of cellobiose or glucose . Thus the induction of cellulase formation seems to be different depending upon the organism tested. Thus, Mandels and Reese (1960) found that *Trichoderma viride* produced more cellulase when cultured on lactose than on cellobiose or glucose . BeMiller *et al.* (1969) pointed out that cellulase was produced by some fungal species in the presence of D-glucose as the sole carbon source and they reported that the enzyme remains attached to the hyphae of *Diplodiazeae* . With respect to protease production, data in table (3) indicated that the yield of protease was highest upon using sucrose followed by lactose and glucose as carbon sources . These results are similar to those of Emtesva (1975) on *B. subtilis* .Such results agreed with those reported by Saad , M. (1995) in *St. venezulae* and Saad *et al.* (1998) on *B. subtilis* .

Table (3): Effect of various carbon sources on lytic enzymes production *by Streptomyces sp.*

Carbon sources	Enzyme activity (units / mg protein)		
	Chitinase	Cellulase	Protease
sucrose	0	0	21.1
chitin	1699	0	0
cellobiose	0	3.8	0.562
glucose	0.359	1.2	10.2
N -acetylglucosamine	2.82	0	0
lactose	0	0	14.3
Carboxymethyl-glucose	0	23.7	0

-Partial purification of lytic enzymes :

Results in table (4) show that most of chitinolytic and proteolytic activities were recovered at 30 % (w/w) ammonium sulphate level. On the other hand, most cellulytic activity was recovered at 60 % (w/w) saturation. The precipitation step was followed by dialysis against 0.1 M phosphate buffer pH 6.5. This step gave enzyme purification of 3.4, 1.3 and 1.3 folds with reference to the crude culture supernatant (chitinase, protease and reference to the crude culture supernatant (chitinase , protease and cellulase) respectively.

Table (4): Partial purification of lytic enzymes produced by *Streptomyces sp.*

Purification step	Total units (units)	Total protein (mg)	Specific activity (unit s/mg protein)	Recovery %	Purification fold
Crude enzyme *	9380	360	26.1	100	1.0
$(NH_4)_2SO_4$ (30%)	9000	100	90.0	95.9	3.4
Crude enzyme**	1200	93	12.9	100	1.0
$(NH_4)_2SO_4$ (30%)	980	58	16.8	81.6	1.3
Crude enzyme ***	2135	120	17.79	100	1.0
$(NH_4)_2SO_4$ (60%)	1740	73	23.8	81.4	1.3

*chitinase ** protease *** cellulase

- Some properties of partially purified lytic enzymes :-

1-Effect of reaction temperature on the activity of the enzymes

The reaction mixture containing partially purified enzyme (chitinase , cellulase or protease) in phosphate buffer pH 6.5 were incubated at different temperatures ranging between 25-80°C.The results indicated that enzymes were optimally active at 40 , 35 and 35 °C respectively. At lower and higher temperatures the activities decreased.

Determination of optimum pH for activity of the partially purified lytic enzymes

The effect of pH on the lytic enzymes activities were investigated using three buffers 0.1M namely citrate phosphate buffer with pH range 2.5-6.0 , phosphate buffer with pH range 6.0-9.0 , and carbonate bicarbonate buffer between pH range 9.0-11.0 in the reaction mixture with appropriate control. It was found that the lytic enzymes exhibited an optima for their activities in the pH range between pH 6.5-7.0 .

3- Thermal and pH stability .

Thermal stability of lytic enzymes (chitinase , cellulase and protease) were examined after incubating enzymes solutions in phosphate buffer pH 6.5 at different temperatures (0-100° C) for 1 hr. It was found that 50% and 65% of chitinase activity ;40%and50% of cellulase activity and 50% and 70 % of protease activity were lost after incubation at 60° C and 70° C respectively. Furthermore the three enzymatic activities were totally lost after incubation for 1 hr. at 80° C or higher.

On the other hand, lytic enzymes containing (chitinase or cellulase or protease) were tested for pH stability during 24 hr. of incubation at pH range of 2.5- 10.0 at room temperatures. It seems that the enzymes were stable 6.0-7.0 but at pH 8.5 the activity was minimally damaged for the three enzymes.

Lysis of some pathogenic fungi with *Streptomyces* sp. :-

The ability of *Streptomyces* sp. to lyse fungal mycelia was investigated by growing the *Streptomyces sp.* in the basal salt medium containing dead fungal mycelia as a sole carbon source was investigated. The cultures were allowed to grow and the residual of the lysed mycelia and lytic activities were recorded. Data in table (5) indicate that the *Streptomyces* sp. could grow and utilize mycelia of *S. rolfsii* and *R. solani* as a carbon source. The percentage of lysis reached 97 %, 86.8 % for *S. rolfsii* and *R. solani* after 4,5 and 2 days respectively. The supernatant of the culture containing mycelia of *S. rolfsii* and *R.. solani* gave 890 and 701 units/ mg protein chitinase activity respectively. The cellulase activity reached 19.2 units/ mg protein in case *S. rolfsii* only , whereas no cellulase activity was detected in case of *R. solani* containing culture. On the other hand protease activities amounted to 11.2; 3.5 units/ mg protein in the supernatant in the two cultures respectively. Our results agree in general with those reported by (Hamed 1992) who indicated that *Streptomyces* sp. has relatively strong lytic activity towards most of the tested fungi . The variation in the lytic activity, however, may be related to the distinctive chemical structure of the fungal cell wall . Among the more representative lytic enzymes involved in fungal cell wall lysis are β-1,3 glucanases, chitinase , cellulase , protease , glucuronidases (Villanueva, 1966 ; Peberdy, 1979 ; Hamed 1996 ; Brinda, M.; and Crawford , L.1997) .

Table (5): Lysis of *S. rolfsii* and *R. solani* a tic activities with *Streptomyces* sp.

Dead Fungal mycelia	Dry Weight of mycelia residue after 72 hr.of growth g/50ml medium (10%w/v)	% of lysis	E	activity (units /mg protein)	
			Ch	cellulase	protease
S. rolfsii	0.16	97		19.2	11.8
R. solani	0.69	86.6		0	3.5
Control *S. Rolfsii*	5.2	0		0	0
R. solani	5.2	0		0	0

Control was used as a dry weight mycelium in two pathoger /50ml

-Effect of different concentrations of *S. rolfsii* an *olani* mycelia on the extent of lysis and lytic activity of *Strep es* sp. :-

In this experiment the concentrations of *S ii* and *R. solani* dead mycelium were varied between 5%-20% added t medium as a carbon source , inoculated with the culture of *Streptomy* . The cultures were incubated on the shaker for 72 hr and the eff of various mycelium concentrations on lysis and lytic activity were mea d . Data presented in table (6) indicate that in the case of *S. rolfsii* the c trations of 5% ,10 % and 15 % were highly lysed and resulted in pr on of high enzymatic activity particularly in chitinase level, whereas 20 celium concentration decreased the lysis extent. In case of *R. ,solani* 5 10% concentrations were completely lysed and higher enzymatic activit e obtained. However lysis of fungal mycelium decreased with i sing the mycelium concentration This was attributed by several au o the increase of the end product that depress, further lysis of the remai nycelia (Monreal and Reese 1969 ; Morrissey *et al.* 1976 and Hamed 19

-Effect of initial pH of medium on lysis of *S. rolf* d *R . solani* by lytic enzymes with *Streptomyces* sp :

The Effect of initial pH of medium on ly mycelium is shown in table (7). The presented data indicate that up creasing initial pH of medium progressive increase in the levels of lytic e es was evident up to pH 7.0 where maximum enzyme levels were att . At higher pH values notable decreases in the enzymatic activities we orded . Nevertheless the mycelium lysis occurred at both acidic and alka H

Table (6): Effect of different concentrations of mycelia of *S. rolfsii* and *R. solani* on production lytic enzymes by *Streptomyces* sp.

Concentration of dry weight mycelium %	Lytic activity after 72 h (units / mg protein)					Lytic activity after 72 h (units / mg protein)				
	Weight of myceli-um residue	% of lysis	*S. rolfsii*			Weight of mycelium residue	% of lysis	*R. solani*		
			chitinase	cellulase	protease			chitinase	cellulase	protease
5	0.013	99.6	720	10.3	10.0	0.029	99.3	630	0	1.5
10	0.021	99.5	715	14.0	10.8	0.048	98.8	622.376	0	2.4
15	0.028	99.3	600	18.9	11.2	1.93	54.0	413	0	3.9
20	2.739	34.7	120	18.0	11.0	3.09	26.4	302	0	2.7

Control was used as a dry weight mycelium of the two pathogens =4.2g/50ml

Table (7): Effect of initial pH values of medium on lysis of *S. rolfsii* and *R. solani* by lytic enzymes with *Streptomyces* sp.

Initial pH	Lytic activity after 72 h (units / mg protein)					Lytic activity after 72 h (units / mg protein)				
	Weight of mycelium residue	% of lysis	*S. rolfsii*			Wight of mycelium residue	% of lysis	*R. solani*		
			chitinase	cellulase	protease			chitinase	cellulase	protease
5.0	0.08	98.3	590	8.0	7.3	0.094	97.7	320	0	2.8
6.0	0.03	99.2	630	9.3	8.6	0.067	98.4	376	0	3.8
6.5	0.0	100	720	10.9	9.9	0.0	100	410	0	5.2
7.0	0.0	100	750	13.0	11.7	0.02	99.9	403	0	5.9
7.5	0.046	98.9	700	14.5	11.0	0.048	98.85	400	0	3.7
8.0	0.049	98.83	680	13.8	10.4	0.053	98.74	395	0	2.9
8.5	0.053	98.8	538	10.9	10.0	0.055	98.6	366	0	2.0
9.0	0.059	98.59	410	8.0	9.6	0.062	98.52	278	0	2.0

Control was used as a dry weight mycelium of the two pathogens =4.2g/50ml

-Lysis of some pathogenic fungi with *Streptomyces* sp. in soil :-

The *Streptomyces* sp. was tested for their ability to secrete the hydrolytic enzymes mentioned above when grown on the pathogen's mycelium, which was previously grown in autoclaved soil. While growing on *S. rolfsii* or *R.solani* culture in soil , the *Streptomyces* sp was inoculated ($2x10^6$ spores /ml) into 5 days- old cultures of the pathogens. Data table (8) indicate that the *Streptomyces* sp. produced more chitinase activity that were superior to cellulase and protease activities .

Table (8): Enzymatic activity of *Streptomyces* sp. grown on mycelium of plant pathogenic fungi (*in vivo*) in soil

Enzymes	Enzyme activity (units / mg protein) Plant pathogen fungus	
	S. rolfsii	R. solani
Chitinase	720	630
*control	0	0
Cellulase	15.9	0
*control	0	0
Protease	11.8	4.2
*control	0	0

***Streptomyces* sp. was inoculated with 2 $x10^5$spores/ml into 5 days-old cultures of the pathogen which had been grown in autoclaved soil**
*** control without pathogens (*S. rolfsii or R. solani*)**

These results suggested that *Streptomyces* sp. lytic enzymes activities may prove to be a possible biological activity against wilt diseases caused by *S. rolfsii* or *R.solani* , such a suggestion requires further studies on viable mycelia and spores of the pathogen. These results have been confirmed by many authors (Hadar *et al.* 1979 ; Tronsmo and Harman, 1992 ; Lorito *et al.* 1993 ; El-Barougy, 1997 ;Rodriguez-Kabana and curi 1978; Elad and Henis 1981a;Elad *et al.* 1982 and Hamed *et al.* 1998) .This phenomenon was found to be correlated with the ability to *Streptomyces* sp isolate to control soilborne diseases under greenhouse conditions .

These differences may also explain the variability in the antagonistic activity of *Trichoderma harzianum* isolates (Elad and Henis 1981b) . It might serve as a tool for an efficient screening of highly parasitic *Streptomyces* sp isolate from soil .

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تحليل الفطريات الممرضة للنبات بواسطة الاستربتوميسس

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فى هذة الدراسة تم عزل سلالة استربتوميسس من التربة المصرية لها القدرة على انتاج بعض الانزيمات مثل انزيمات الكيتينيز و السليولييز والبروتييز من بيئة تحتوى على الكيتن أو كربوكسى ميثيل سليلوز أو الكازين كمصدر للكربون أو تحتوى على الخلايا الميتة للفطريات الممرضة للنبات مثل فطرى اسكلوروشيم رولزفاى والريزوكتونيا سولانى فى البيئة وذلك عند تنميتها على درجة حرارة ٢٨°م كذلك تم دراسة انسب درجة أس هيدروجينى فى البيئة حيث وجد أن درجة ٥و٦-٠و٧ هى افضل درجة اس هيدروجينى تعطى عندها الأستربتوميسس اعلى انتاجية من هذة الانزيمات المحللة كذلك تم دراسة تأثير التركزات المختلفة من الفطران على انتاج هذة الانزيمات المحللة لهما و دراسة تأثير اختلاف درجة الأس الهيدروجينى فى البيئة على تحلل هذان الفطران وتم فصل وتنقية هذة النزيمات تنقية جزئية بواسطة كبريتات الامونيوم ثم دراسة العوامل المؤثرة على نشاط هذة الانزيمات تمهيدا لدراسة تأثير زراعة الاستربتوميسس فى تربة تحتوى على هذانالفطران فى صورتهما الحية حيث وجد ان الاستربتوميسس لها القدرة على النمو وانتاج الانزيمات المحللة للفطريات الممرضة للنبات عند وجودها فى صورة حية فى التربة وذلك تمهيدا لتطبيق هذة النتائج على مستوى الحقل .