Journal of Environmental Sciences, 2010; Vol. 39 : 15-31

PRODUCTION OF CELLULASE-FREE XYLANASE FROM A LOCALLY ISOLATED *STREPTOMYCES PSEUDOGRISEOLUS*

Mansour, F. A.[1]; Mohamed S. Abdel-Aziz[2]; Azza, A. AbouZeid[3]; Mohamed, Fadel[2] and Ahmed, S. Abdel-Razik

[1]*Botany Department, Faculty of Science, Mansoura University, Mansoura, Egypt*

[2]*Microbial Chemistry Department, National Research Center, Dokki, Cairo, Egypt*

[3]*Botany Department, Faculty of Science. Zagazig University, Zagazig, Egypt*

ABSTRACT

A total of 91 strains of actinomycetes were isolated from compost and soil from Aga city. These isolates were tested for their ability to produce xylanase qualitatively using three different screening tests and the strains that showed positive results were tested quantitatively to select the potent xylanase producer. Three isolates, two from compost (9C and 11C) and one isolate from soil (34S), were found to produce highest xylanase activity. These three isolates were identified as Streptomyces pseudogriseolus, S. werraensis and S. griseoflavus. S. pseudogriseolus was selected for its highest cellulase–free xylanase activity. The enzyme production from this strain was maximized by optimizing the environmental and nutritional conditions. The crude enzyme showed an optimum activity at pH6 and showed pH stability over the range from 5 to 11. This enzyme also showed an optimum temperature of 60^{o}C and thermally stable up to 40^{o}C and retained 65% of its activity at 50^{o}C and totally inactivated beyond 60^{o}C.

Key words: *Xylanase, Streptomyces, identification, production and characterization*

INTRODUCTION

Hemicelluloses including xylans are the second most abundant natural polysaccharide following cellulose (Collin et al., 2005). These compounds are present in the cell wall and in the middle lamella of plant cells. Agricultural residues contain 20- 40% hemicelluloses formed by pentose sugars (Magge and Kosaric, 1985). Monomers of various hemicelluloses are useful in the production of different antibiotics, alcohols, animal feed and fuels (Thompson, 1983). Hemicelluloses consist of a mixture of hexosans, pentosans and polyuronides. Xylan is the most known type of the hemicelluloses. It has a linear backbone of β-1,4-linked D-xylopyranose residues that often has side chains of O-acetyl, arabinosyl and methylglucuronosyl substituents (Blanco et al., 1997; Maheshwari et al., 2000).

There is great interest in the enzymatic hydrolysis of xylan due to possible applications in feed stock, chemical production and paper manufacturing (Coughlan and Hazlewood, 1993). Endo-β-1,4-xylanase (1,4-β- D-xylan xylanohydrolase: E.C. 3.2.1.8) is the main enzyme responsible for the cleavage of the linkages within the xylan backbone (Belfaquih et al., 2002). Xylanases are widely distributed in many species of bacteria (Yang et al., 1995;

Roy, 2004; Pason et al., 2006) and fungi (Knob and Carmona, 2008; Antoine et al., 2009; Gupta et al., 2009), (Wong et al., 1988; Senior et al., 1989; Eriksson et al., 1990).

Actinomycetes play a significant role in recycling of nutrients and involve in the primary degradation of organic matter in compost and related materials (Sykes and Skinner, 1973). They are considered as an important source of enzymes involving in lignocelluloses degradation and activity against xylan (McCarthy, 1987). Actinomycetes xylanase activity is largely known from studies on *Streptomyces* spp. (Yasui, et al., 1988; Ball and McCarthy, 1989) and *Thermomnospora* spp. (Stutzenberger and Bodine, 1992).

This investigation was undertaken to isolate actinomycetes which are capable of producing high xylanase activity and to, identify the best produces and optimizing the culture conditions for the enzyme production. Also, the characterization of the crude enzyme from the best producer was studied.

MATERIALS AND METHODS

Isolation of actinomycetes :

An air dried samples of compost and soil (50gm of each) were dissolved separately in 200ml of sterile distilled water and subjected to shake on a reciprocal shaker for 2 hr. A serial dilution has been done (10^{-1} to 10^{-6}) and then used to inoculate the isolation medium. Starch-nitrate agar plates supplemented with the anti-fungus nystatin (50U/ml) and the anti-bacterium nalidixic acid (20mg/ml) (Seong et al., 2001) were inoculated with 100 ml from the different dilutions and incubated at 30°C for 7-10 days and the appropriate plates were used for the isolation of single actinomycete colonies.

Qualitative screening of xylanase enzyme :

Actinomycete isolates from both soil and compost were screened qualitatively on agar plates containing the medium adopted by Wang et al. (2003) which has the following ingredients (%): xylan (from oat spelt), 0.25; $NaNO_3$, 0.1; K2HPO4, 0.1; $MgSO_4$.7H2O, 0.05; yeast extract, 0.05 and agar, 2.0. The pH was adjusted to about 7.0. Plates were incubated at 30°C for 3 days. Xylanase activity was checked by the formation of clear zone around growth, staining the plates with 0.5% Congo red for 15min. then destained with 1M NaCl or by flooding the plates with absolute ethanol for 16 h at room temperature.

Enzyme production :

The isolate to be selected was cultivated in 250 ml-capacites Erlenmyer flasks each containing 50ml medium composed of (g/l): xylan, 10.0; $NaNO_3$, 1.5; KH_2PO_4, 3.0; K_2HPO_4, 6.0; $MgSO_4$. $7H_2O$, 0.2; $CaCl_2$, 0.05; $MnSO_4.7H_2O$, 0.01; $ZnSO_4$.7H2O, 0.001 and 1000ml distilled water. The pH was adjusted to 7 (Nascimento et al., 2003). The cultures were incubated for 6 days at 30°C on a rotary shaker (150 rpm) after inoculation with 5ml of a dense spore suspension. The cultures were then centrifuged at 5000 rpm for 20min. using a cooling centrifuge (Jouan E956) and xylanase activity was measured in the culture supernatant. CMcase, FPase and soluble protein were also measured.

Taxonomic identification of the experimental isolates

To identify these strains, the ISP methods were applied (Shirling and Gottlieb, 1966), and the diagnostic properties of the strain were compared with those reported for type culture of Streptomyces species according to ISP (Shirling and Gottleib (1968a, 1968b and 1969 and 1972), and the strains were rather identified using Bergey's Manual of Systematic Bacteriology (1989) and Bergey's Manual of Determinative Bacteriology (1994).

Enzymes and protein assays :

The enzyme assay has been measured as described by Xiong et al. (2003) as follows: 1% xylan (from oat spelts) was dissolved in 0.05M acetate buffer (pH 5.0) for 15 min at 50oC. The released reducing sugars were determined by the method of Miller (1959). Xylanase unit is the amount of enzyme that liberates 1m mole of reducing sugar (measured as xylose) per min. Carboxymethylcellulase (CMCase) and FPase (C_1 cellulase) were measured according to Mandels et al. (1974) by adding 0.5ml of 1.0% of Carboxymethylcellulose (CMC) in citrate buffer (0.05M) with a pH value of 4.8. The mixture was incubated at 50^{o}C for 30 min. FPase was assayed by mixing 1ml of 0.05M citrate buffer (pH 4.8) and 50mg of Whatmann filter paper no.1 (strips of 1x6cm) at 50^{o}C for 1 hour. Reducing sugars from both CMCase and FPase were measured as glucose according to the method of Miller (1959). Protein was tested according to Lowry et al. (1951).

RESULTS

Screening studies on xylanase production from isolated actinomycets

A number of ninty-one (fifty-two isolates from compost and thirty-nine isolates from soil) related to actinomycetes were isolated separately from compost and soil samples form Aga city (Mansoura, Egypt) using starch nitrate medium supplemented with both nystatin and nalidixic acid (Seong et al., 2001). These isolates were tested for xylanase activity qualitatively using xylan-agar plates, Congo red stained xylan-agar plates and ethanol (99.9%) flooded xylan-agar plates. All the strains isolated from compost showed xylanase activity except 3 isolates. On the other hand, only 23 strains of soil isolates showed xylanase activity. Fig. 1 illustrates the best xylanase positive results (formation of clear zone) when isolates 9C (compost), 11C (compost) and 34S (soil) were grown on xylan-agar plates (A1, B1 and C1), Congo red stained xylan agar plates (A2, B2 and C2) and ethanol flooded xylan agar plates (A3, B3 and C3), respectively. Quantitative studies for xylanase activity for the three isolates revealed that isolate no. 9C showed highest celluose-free ylanase and therefore, it was chosen for further studies. Table 1 summarized the enzyme activities (xylanase, CMCase and FPase) of the best xylanase producers (9C, 11C and 34S). This has been established using liquid medium containing xylan as a sole carbon source (0.2%). Xylanase, CMCase and FPase activities were assayed after 6 days of incubation on a rotary shaker (150rpm).

Taxonomical study of the experimental isolate :

Cell wall analysis : The whole cell hydrolysate was examined to determine the isomers of diaminopimelic acid (DAP) and sugar pattern of isolates following the methods of Beck-

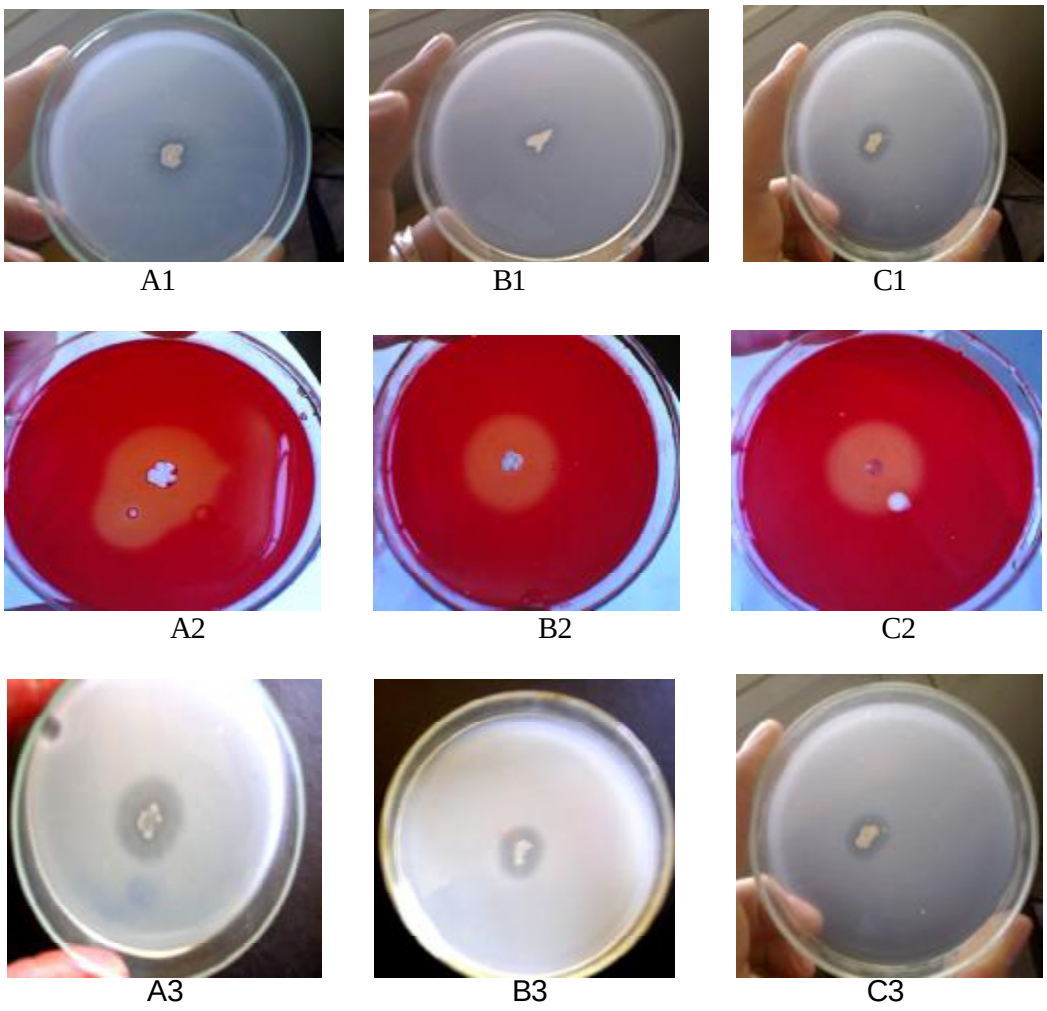


Figure (1). Qualitative screening of xylanase-producing isolates 9C, 11C and 34S when grown on xylana agar plates (A1, B1 and C1), Congo red stained xylan agar plates (A2, B2 and C2) and xylan agar plates flooded with ehanol (A3, B3 and C3), respectively.

Table (1) Quantitative screening of xylanase activity from potent xylanase producing streptomycetes isolates from compost and soil:

Isolate no.	Final pH	Protein (mg/ml)	Xylanase		Cellulases			
			Activity (U/ml)	Specific activity (U/mg protein)	CMCase		FPase	
					Activity (U/ml)	Specific activity (U/mg protein)	Activity (U/ml)	Specific activity (U/mg protein)
9C	6.6	0.176	9.24	52.50	-	-	-	-
11C	6.7	0.181	9.18	50.72	-	-	-	-
34S	6.5	0.179	8.96	50.06	0.60	3.35	0.13	0.73

er et al, 1965) and Lechevalier and Lechevalier (1970). The chromatographic analysis of the cell wall hydrolysate revealed that the three isolates contain LL-DAP and no characteristic sugars, and was, therefore, classified belong to the Genus Streptomyces.

Morphological characteristics: Table (2) revealed the taxonomical results of the best three xylanase producing Streptomyces isolates. From this table all the three isolates showed open spiral spore chains (Fig.2 a, b &c) with smooth surface for isolate no. 9C and spiny spore surfaces for both isolates 11C and 34S (Fig. 3 a, b&c). Aerial mass is white in color-series for both 9C and 11C but grey for 34S when grown on yeast-malt agar, oatmeal agar, salts-starch agar and glycerol asparagines agar. No distinctive or diffusible pigments were found with the previous media. Melanoid pigment was not produced. These strains were deposited in the microbial chemistry department collection, NRC, Egypt.

Optimization of xylanase production from Streptomyces

Table (2) Morphological, physiological and chemotaxonomical results of streptomycetes isolates that showed maximum xylanase production:

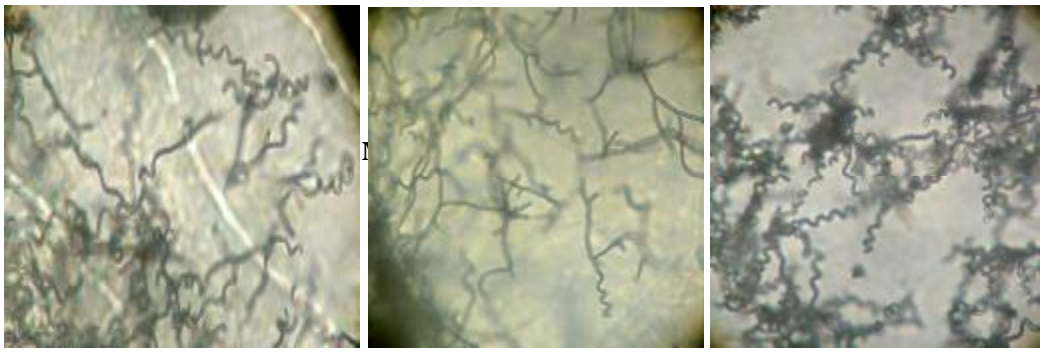
Isolate no.	Spore chain morphology	Spore surface	Color of colony	Reverse side of colony	Diffusible pigments	Melanoid pigment production
9C	Open spiral	Smooth	White	Yellow brown	-	-
11C	Open spiral	Spiny	White	Yellow brown	-	-
34S	Open spiral	Spiny	grey	Yellow brown	-	-

Table (2) continued

Isolate no.	Enzyme activities			Pectin decomposition	Chitin decomposition	Nitrate reduction	H_2S production	Xanthine degradation
	Proteolysis	Lipolysis	Lecithinase					
9C	-	-	-	+	-	-	+	-
11C	-	-	-	+	-	-	+	-
34S	-	-	+	+	-	-	-	-

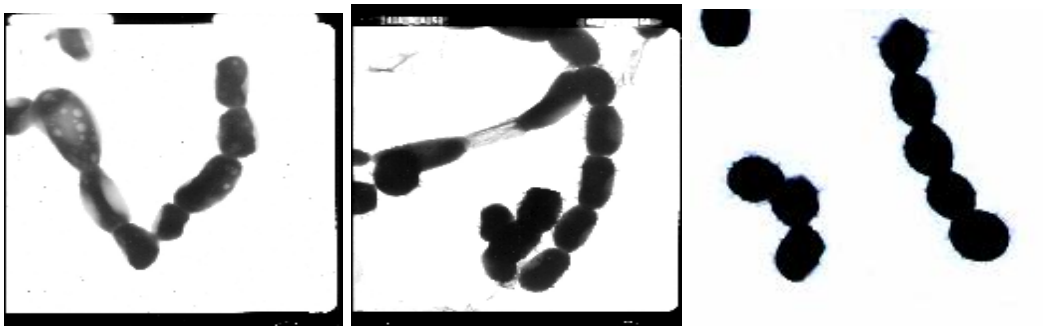
Table (2) continued

Utilization of different carbon sources										
-ve control	**+ve control (glucose)**	**Arabinose**	**Fructose**	**Rhamnose**	**Sucrose**	**Mannitol**	**Xylose**	**Raffinose**	**Inositol**	**Galactose**
+	+	+	+	+	-	+	+	+	+	+
+	+	+	+	+	-	+	+	-	+	+
+	+	+	+	+	-	+	+	-	+	+



a b c

Figure (2). (a, b & c): Photographs showing spore chain morphology of streptomycete isolates no.9, 11 and 34, respectively (X400)



a b c

Figure (3) (a, b & c): Transmittance electron micrography (TEM) of spores of streptomycete isolates no. 9, 11 and 34, respectively (X 20,000).

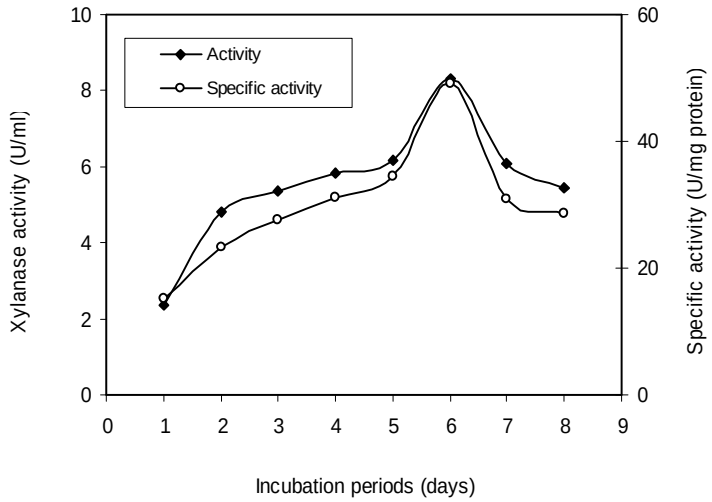


Figure (4) Effect of incubation periods (days) on xylanase production by *Streptomyces pseudogriseolus* using corn comb xylan.

Effect of incubation period :

The results presented in Fig. 4 revealed that there was an induction phase of xylanase enzyme in the first 24 h during which little xylanase was synthesized. It was indicated by an activity of 2.38 U/ml and specific activity of 15.16 U/mg protein respectively. Following this phase, the enzyme production increased gradually reaching its highest degree (8.32 U/ml, 49.23 U/mg protein) after 6 days of growth.

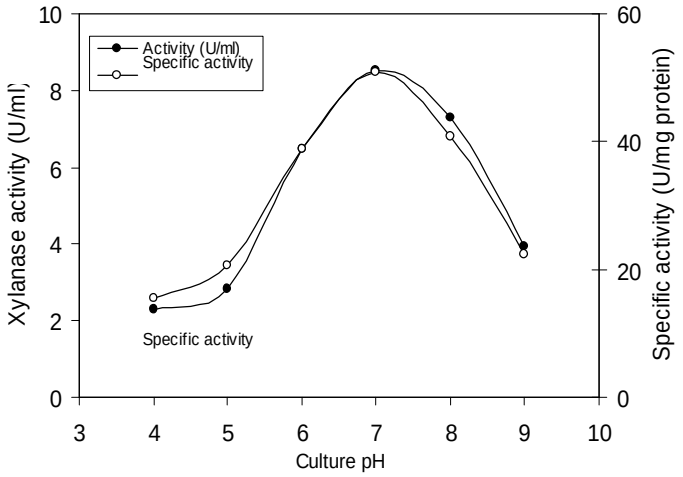


Figure (5) Effect of the culture initial pH on xylanase production by *Streptomyces pseudogriseolus*.

Effect of initial pH :

The effect of initial pH of the fermentation medium on xylanase production by Streptomyces pseudogriseolus was investigated. The results presented in Figure (5) revealed that the initial pH of 7 showed maximum enzyme activity (8.51 U/ml and 50.96 U/mg protein).

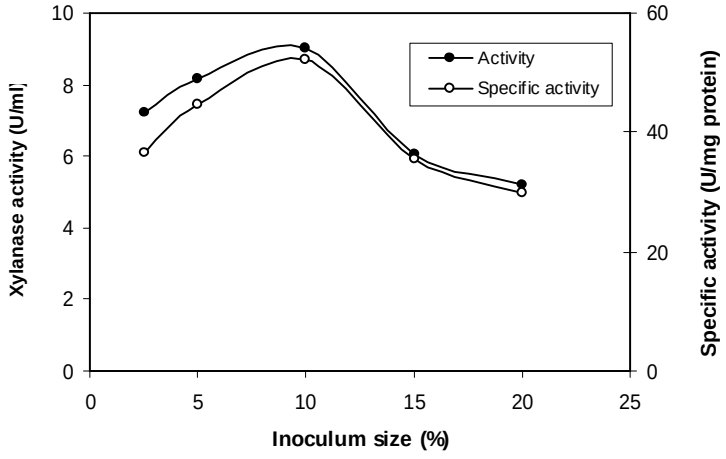


Figure (6) Effect of inoculum size on xylanase production by *Streptomyces pseudogriseolus*.

Effect of inoculum size

An inoculum percent of 10% from previously prepared spore suspension ($2.51x10^9$ cell /ml) had a great effect on xylanase production from the tested strain (Fig. 6). 10% of the prepared stock showed maxiumum xylanase activity (9.02U/ml) and a specific activity of 52.14U/mg protein.

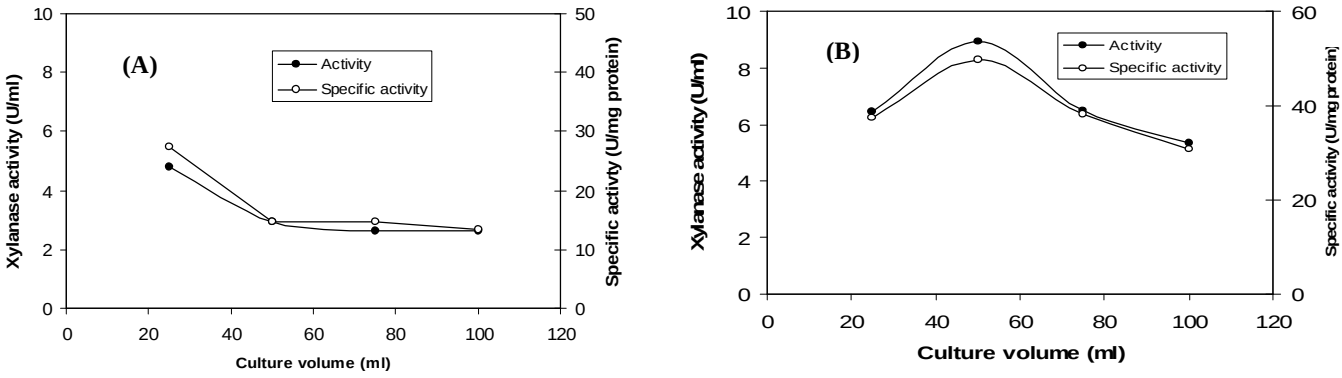


Figure (7) Effect of medium volume on the activity of xylanasee enzyme produced from *Streptomyces pseudogriseolus. A:* (Static culture). B: (Shaken culture).

Effect of medium volume/flask :

The results presented in Figs. 7 (A & B) revealed that the highest level of xylanase activity was noticed in shaken culture with a medium volume of 50 ml/flask (250-ml). It reached 8.92U/ml with a specific activity of 49.83U/mg protein. Enzyme synthesis by the streptomycete under investigation needs high aeration. Furthermore, decrease in the enzyme levels have been observed with the increase in medium volume accordingly, the decrease in aeration rate. On the other hand, the production of xylanase enzyme was greatly lowered in cultures incubated without shaking (static cultures). The activity was 4.80 U/ml in a static culture of air: medium ratio of 9:1. The phenomenon that was observed in static cultures may be related to secretion of other metabolites by the isolate that affected both growth and enzyme denaturation

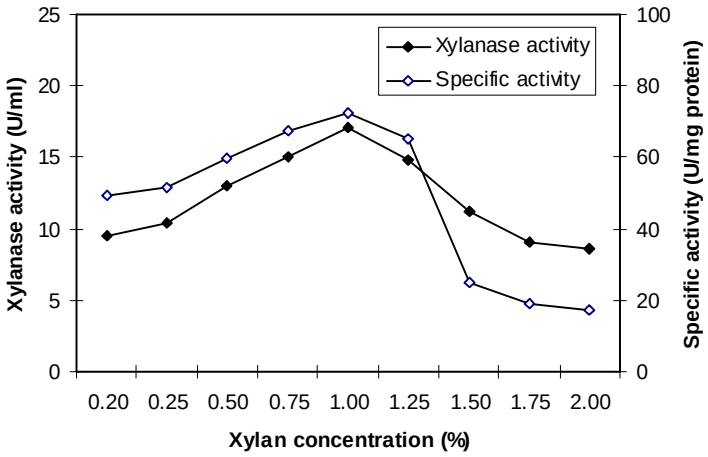
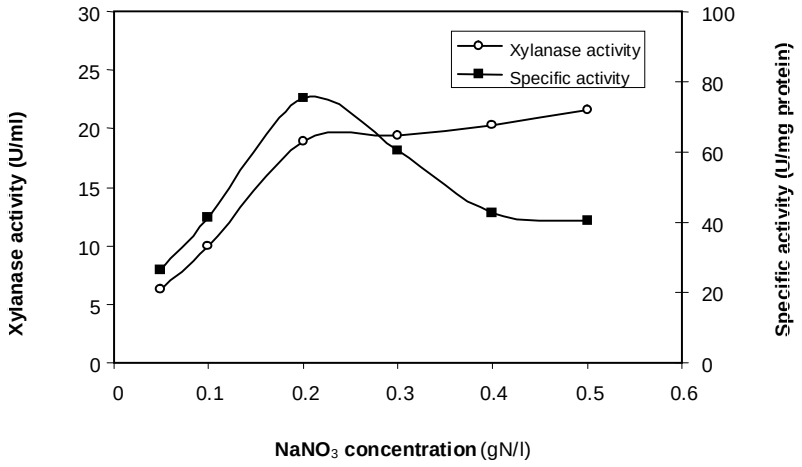


Figure (8) Effect of different xylan concentrations on xylanase production from *Streptomyces pseudogriseolus.*

Effect of different carbon sources :

It was found that xylan was the only carbon source stimulate xylanase production. Virtually, no enzyme activity could be detected in the case of the other carbon sources tested (CMC, cellulose, dextran, maltose, sucrose, fructose, glucose, galactose, xylose arabinose). Also CMCase and FPase were also tested and the results showed that no cellulose activity was produced with any of these carbon sources. A xylan concentration of 1% (w/v) showed maximum xylanase production (17.12U/ml) with a specific activity of 72.54U/mg protein (Fig. 8).



Effect of nitrogen sources :

The optimal concentration of sodium nitrate (1.2g/l or 0.2gn/l) in the basal medium was replaced in nitrogen equivalent by different nitrogen sources. From the inorganic and organic nitrogen sources tested it was found that sodium nitrate was the best source for xylanase production with an activity of 20.34U/ml and specific activity of 78.54U/mg protein (Table 3). Fig. 9 demonstrates the effect of different concentrations of sodium nitrate (best nitrogen source) on xylanase production by Streptomyces pseudogriseolus. It was found that $NaNO_3$ in a concentration of 1.2g/l showed maximum xylanase production (19.94 U/ml and 77.29 U/mg protein).

Characterization of crude xylanase :

Optimum pH: Xylanase from Streptomyces pseudogriseolus, exhibited an optimum pH of 6 (Fig.10).

pH stability: Xylanase from Streptomyces pseudogriseolus, was stable over a pH range from 5 to 11, and at pH 12; 30% enzyme loss had been detected but at pH 13 there was no activity (Fig.11).

Optimum temperature: Results in Figure (12) revealed that xylanase enzyme showed an optimum at 60^{o}C.

Thermal stability: The enzyme was ther-

Table (3) Effect of different nitrogen sources on xylanase production from *Streptomyces pseudogriseolus.*

Nitrogen source	Final pH	Protein (mg/ml)	Activity (U/ml)	Specific activity (U/mg protein)
$NaNO_3$	7.21	0.259	20.34	78.53
NH_4Cl	6.72	0.404	14.22	35.20
KNO_3	7.43	0.365	13.18	36.11
NH_4NO_3	6.94	0.689	20.46	29.69
$Ca(NO_3)_2$	6.62	0.319	13.32	41.76
$(NH_4)_2SO_4$	6.91	0.312	20.24	64.87
$NH_4H_2PO_4$	6.96	0.283	17.82	62.67
$(NH_4)_2HPO_4$	8.12	0.385	9.92	25.77
$(NH_4)_3PO_4$	7.53	0.275	14.36	52.23
Urea	7.35	0.489	10.48	21.43
Peptone	7.28	0.506	6.20	12.26
Casein	7.23	0.578	8.60	14.88
Yeast extract	7.18	0.733	16.88	23.03

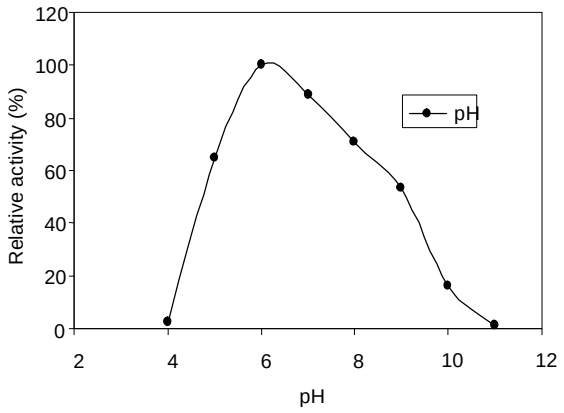


Figure (10) pH profile of crude xylanase enzyme from *Streptomyces pseudogriseolus.*

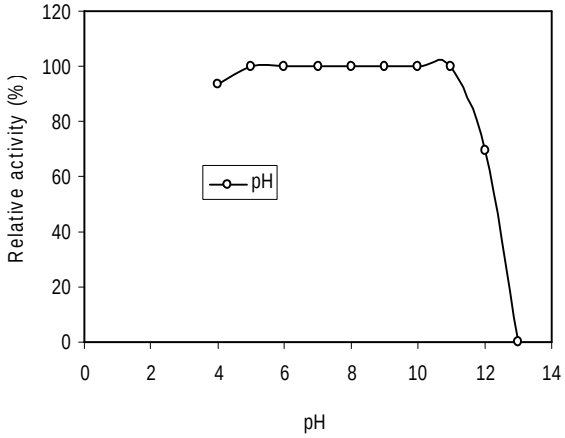


Figure (11) Determination of the pH stability of xylanase enzyme from *Streptomyces pseudogriseolus.*

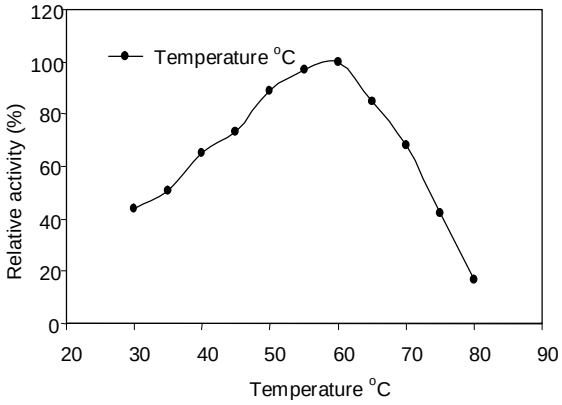


Figure (12) Optimum temperature of crude xylanase enzyme from *Streptomyces pseudogriseolus.*

mally stable up to 40°C and showed 35% activity loss at 50°C but at60°C the enzyme retained 10% of its maximum activity whereas, it lost its activity at 70°C (Fig.13).

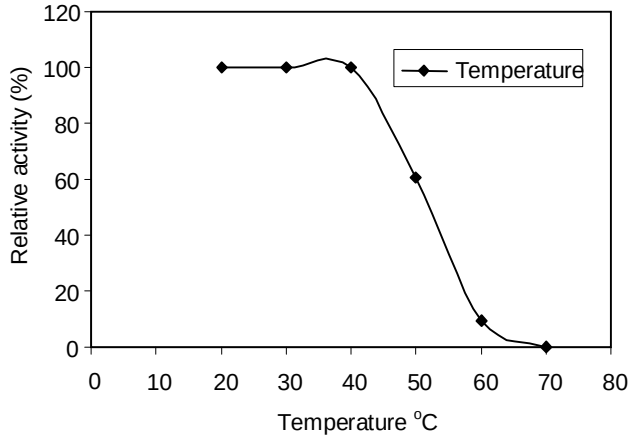


Figure (13) Thermal stability of crude xylanase enzyme from *Streptomyces pseudogriseolus.*

DISCUSSION

A total of 91 strains belonging to actinomycetes were isolated from both compost and soil at Aga city (Mansoura, Egypt) using starch-nitrate medium provided with nalidixic acid (anti-bacterium) and nystatin (anti-fungus) as described by Seong et al. (2001). These isolates were screened qualitatively for their xylanase activity using three visual methods. The first method depends on the formation of clear zones around the microbial growth on xylan-agar plates after 3 days (Roy and Zainul-Abedin, 2002; Wang et al., 2003), staining similar xylan-agar plates with an aqueous solution of Congo red (0.5%) after the same incubation period (Teather and Wood, 1982; Gessesse and Gashe, 1997; Ninawe et al., 2006) or flooding xylan-agar plates with absolute ethanol after the same growth period (Rawashdeh et al., 2005; Saadoun et al., 2007). Isolates that showed positive results were screened quantitatively using broth medium containing xylan (0.2%) as sole carbon source. The highest three xylanase producing isolates (two from compost, 9C & 11C and one from soil, 34S) were taxonomically identified as Streptomyces pseudogriseolus, S. werraensis and S. griseoflavus, respectively. *Streptomyces pseudogriseolus* was selected for further studies as it had the highest cellulose-free xylanase.

Streptomyces pseudogriseolus was found to produce higher xylanase level after 6 days

with best initial pH of 7 using 250ml-Erlenmeyer conical flasks each had 50ml culture medium. These results are analogus to somehow to that found by Tsujibo et al. (1992). Xylan was found to be the sole carbon source responsible for enzyme production with a best concentration of 1%. Elegir et al. (2009) found that xylan (from oat spelt) at this concentration gave the best xylanase enzyme from *Streptomyces pseudogriseolus* strain B-12-2. On the other hand, sodium nitrate at a concentration of 1.2g/l gave optimum xylanase production (Nascimento et al., 2003)

Crude xylanase enzyme from *Streptomyces pseudogriseolus* exhibited an optimum pH of 6 and this result was similar to that deduced from several Streptomyces strains (Kluepfel et al., 1990; Patel and Ray, 1994; Georis et al., 2000). This enzyme was stable over a pH range from 5 to 11 and this result is somewhat near to that investigated by Tsujibo et al. (1992). The temperature profile for this enzyme was also studied. This enzyme showed an optimum temperature range from 55 to 60^{o}C with maximal activity at 60^{o}C. These findings were comparable to that described by Flores et al. (1997), Beg et al. (2000) and Morosoli et al. (1986) or to someextent lower than that found by Nascimento et al. (2002). The thermal stability of this enzyme was also investigated from room temperature to 80^{o}C and it was found that the enzyme conserved most of its activity up to 50^{o}C. However, incubation of the enzyme for 1h at temperatures over 50^{o}C inactivated it. These observations were equivalent to that had been found by Ruiz-Arribas et al. (1995)

REFERENCES

Antoine, A. A.; Jacqueline, O. and Thonart, R. (2009) : Xylanase production by Penicillium canescens on soya oil cake in solid-state fermentation. Appl. Biochem Biotechnol (In press)

Ball, A. S. and McCarthy, A. J. (1989) : Production and properties of xylanases from actinomycetes. J. Appl. Bacteriol. 66: 439-44.

Beg, Q. K.; Bhushan, B.; Kapoor, M. and Hoondal, G. S. (2000) : Production and characterization of thermostable xylanase and pectinase from Streptomyces sp. QG-11-3. J Ind Microbiol Biotechnol. 24:396-402.

Belfaquih, N.; Jaspers, C.; Kurzatkowski, W. and Penninckx, M. J. (2002) : Properties of Streptomyces sp. Endo-b-xylanases in relation to their applicability in kraft pulp bleaching. World J. Microbiol. Biotechnol. 18: 699-705.

Bergey's Manual (1994) : Bergey's Manual of Determinative Bacteriology: Holt J. G.; krieg, N. R.;Sneath,P.H.A.;Staley, J.T. and Williams, S.T., 9th ed.,Williams and Wilkins, Baltmore.

Bergey's Manual (1989) : Bergey's Manual of Systematic Bacteriology Vol. 4; section 29; Streptomyces and related genera. Williams and Wilkins Company, Baltimore, U.S.A.

Becker, B.; Lechevalier, M. P. and Lechevalier., H. A. (1965) : Chemical composition of cell-wall preparation from strains of various from-genera of aerobic actinomycetes. Appl. Microbiol., 13: 236-743.

Blanco, J.; Coque, J. J. R.; Velasco, J. and Martin, J. F. (1997) : Cloning, expression in Streptomyces lividans and biochemical characterization of a thermostable endo-_-1,4-xylanase of Thermomonospora alba ULJB1with cellulose binding ability. Appl. Microbiol. Biotechnol. 48: 208-217.

Collin, T.; Gerday, C. and Feller, G. (2005) : Xylanases, xylanases families and extremophilic xylanases. FEMS Microbiol. Rev. 29: 3-23.

Coughlan, M. P. and Hazlewood, G. P. (1993) : b-1,4-d-xylan-degrading enzyme systems; biochemistry, molecular biology and applications. Biotechnol. Appl. Biochem.,17: 259-289.

Elegir, G.; Szakacs, G. and Jeffries, T. W. (1994) : Purification, characterization, and substrate specificities multiple xylanases from Streptomyces sp. Strain B-12-2. Appl. Environ. Microbiol. 60:2609-2615.

Eriksson, K. E. L.; Blanchette, R. A. and Ander, P. (1990) : Microbial and Enzymatic Degradation of Wood and Wood Components, Springer-Verlag, New York, N.Y.

Flores, M. E.; Pérez. R. and Huitrón, C. (1997) : b-Xylosidase and xylanase characterization and production by Streptomyces sp. CH-M-1035. Lett. Appl Microbiol. 24:410–6.

Georis, J.; Giannotta, F.; Buyl, E. D.; Granier, B. and Frère, J. M. (2000) : Purification and properties of three endo-b-1,4-xylanases produced by Streptomyces sp. strain S38 which differ in their ability to enhance the bleaching of kraft pulps. Enzyme Microb. Technol. 26:178-86.

Gessesse, A. and Gashe, B. A. (1997) : Production of alkaline xylanases by an alkalophilic Bacillus sp. isolated from an alkaline soda lake. J. Appl. Microbiol. 83: 402-406.

Gupta, V. K.; Guar, R.; Gautam, N.,; Kumar, P.; Yadav, I. J. and Darmwal, N. S. (2009) : Optimization of xylanase production from Fusarium Solani F7. Amer. J. Food Technol. 4(1):20-29.

Kluepfel. D.; Vats-Mehta, S.; Aumont F, Shareck F. and Morosoli R. (1990) : Purification and characterization of a new xylanase (xylanase B) produced by Streptomyces lividans 66. Biochem J; 267: 45–50.

Knob, A. and Carmona, E. C. (2008) : Xylanase production by Penicillium sclerotiorum and its characterization. World Appl. Sci. J. 4 (2): 277-283.

Lechevalier, M. P. and Lech. H. A. (1970) : Chemical composition as a criterion in the classification of aerobic actinomycetes. Intr. J. Syst. Bacterial. 20: 425-442.

Lowry, O. H.; Rosebrough, N. I.; Farr, A. L. and Randall, R. J. (1951) : Protein measurement with the Folin phenol reagent. J. Biol. Chem. 193:265-275.

Magge, R. J. and Kosaric, M. (1985) : Bioconversion of hemicellulose. Adv. Biochem. Eng. Biotechnol. 32, 61-93.

Maheshwari, R.; Bharadwaj, G. and Bhat, M. K. (2000) : Thermophilic fungi: their physiology and enzymes. Microbiol. Molec. Biol. Rev. 64: 461- 488.

Mandels, M.; Hortz, L. and Nystrom, J.

(1974) : Enzymic hydrolysis of waste cellulose. Biotechnol. Bioeng. 16:1471-1493.

McCarthy, A. J. (1987) : Lignocellulose-degrading actinomycetes. FEMS Microbiol. Rev. 46: 145-163.

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

Morosoli, R.; Bertrand, J. L.; Mondou, F.; Shareck, F. and Kluepfel, D. (1986) : Purification and properties of a xylanase from Streptomyces lividans. Biochem. J. 239:587-592.

Nascimento, R. P.; Coelho, R. R. R.; Marques, S.; Alves, L..; Gırio, F. M.; Bon, E. P. S. and Amaral-Collaço, M. T. (2002) : Production and partial characterization of xylanase from Streptomyces sp. strain AMT-3 isolated from Brazilian cerrado soil. Enzyme and Microb. Technol. 31:549-555.

Ninawe, S.; Lal, P. and Kuhad, R. C. (2006) : Isolation of three xylanase-producing strains of actinomycetes and their identification using molecular methods. Curr. Microbiol. 53:178-182.

Pason, P.; Kyu, K. L. and Ratanakhanokchai, K. (2006) : Paenibacillus curdlanolyticus strain B-6 xylanolytic-cellulolytic enzyme system that degrades insoluble polysaccharides. Appl. Environ. Microbiol. 72(4): 2483-2490.

Patel, B. and Ray, R . M. (1994) : Production and characterization of xylanase from Streptomyces species grown on agricultural wastes. World J. Microbiol. Biotechnol. 10:599-604.

Rawashdeh, R.; Saadoun, I. and Mahasneh, A. (2005) : Effect of cultural conditions on xylanase production by Streptomyces sp. (strain Ib 24D) and its potential to utilize tomato pomace. Afr. J. Biotechnol. 4 (3):. 251-255.

Roy, N. (2004) : Characterization and identification of xylanase-producing bacterial strains isolated from soil and water. Pak. J. Biol. Sci 7(5): 711-716.

Roy, N. and Zainul-Abedin, M. D. (2002) : Isolation and molecular characterization of xylanase-producing bacteria from drain water. Online J. Biol. Sci. 2(6): 399-402.

Ruiz-Arribas, A.; Fernandez-Abalos, J. M.; Sanchez, P.; Garda, A. and Santamaria, R.. (1995) : Overproduction, purification, andbiochemical characterization of a xylanase (Xys1) from Streptomyces halstedii JM8. Appl. Environ. Microbiol. 61 (6): 2414-2419.

Saadoun, I.; Rawashdeh, R.; Dayeh, T.; Ababneh, Q. and Mahasneh, A. (2007) : Isolation, characterization and screening forfiber hydrolytic enzymes-producing streptomycetes of Jordanian forest soil. Biotechnology. 6(11): 120-128.

Senior, D. J.; Mayers, P. R. and Saddler, J. N. (1989) : Production and purification of xylanases. In: Plant cell wall polymers. (Eds. Lewis, N.G. and Paice, M.G.) Washington D.C. Am. Chem. So. pp. 641-654.

Seong, C. N.; Choi, J. H. and Baik, K.S. (2001) : An improved selective isolation of rare actinomycetes from forest soil. J. Microbiol. 39(1): 17-23.

Shirling, E. B. and Gottlieb, D. (1966) : Methods for characterization of Streptomyces species. Int. J. Syst. Bacteriol. 16:313-340.

Shirling, E. B. and Gottlieb, D. (1968a) : Cooperative description of type cultures of Streptomyces. II. Species descriptions from first study. Int. J. Syst. Bacteriol. 18: 69-189

Shirling, E. B. and Gottlieb, D. (1968b) : Cooperative description of type cultures of Streptomyces. III. Additional species descriptions from first and second studies. Int. J. Syst. Bacteriol. 18, 279-392.

Shirling, E. B. and Gottlieb, D. (1969) : Cooperative description of type cultures of Streptomyces. IV. Species descriptions from the second, third and fourth studies. Int. J. Syst. Bacteriol. 19: 391-512.

Shirling E. B. and Gottlieb, D. (1972) : Cooperative description of type strains of streptomyces. V. Additional descriptions. Int. J. Syst. Bacteriol 22 (4) : 265-394.

Stutzenberger, F. J. and Bodine, A. B. (1992) : Xylanase production by Thermomnospora curvata. J. Appl. Bact., 72: 501-511.

Sykes, G. and Skinner, F. A. (1973) : Actinomycetales: Characteristics and practical importance. Academic Press. London. Pp 339.

Teather, R. M. and Wood, P. J. (1982) : Use of congo red-polysaccharide interactions in enumeration and characterization of cellulolytic bacteria from bovine rumen. Appl. Environ. Microbiol. 43(4): 777-780.

Thompson, N. S. (1983) : Hemicellulose as a biomass resource. In Woodand Agricultural Residues. Research on Use for Food, Fuel and Chemical (ed. Solters, E. J.), Academic Press, Sandiego, pp.101-119.

Tsujibo, H.; Miyamoto, K.; Kuda, T.; Minmai, K.; Sakamoto, T.; Haseawa, T. and Inamori, Y. (1992) : Purification, properties, and partial amino acid sequences of thermostable xylanases from Streptomyces thermoviolaceus OPC-520. Appl. Environ. Microbiol. 58(3): 371-375.

Wang, S. L.; Yen, Y. H.; Shih, I. L.; Chang, A. C.; Chang, W. T.; Wu, W. C. and Chai, Y. D. (2003) : Production of xylanases from rice bran by Streptomyces actuosus A-151. Enzyme Microb. Technol. 33:917-925.

Wong, K. K. Y.; Tan, L. U. L. and Saddler, J. N. (1988) : Multiplicity of beta-1,4-xylanase in microorganisms : functions and applications. Microbiol. Rev. 52, 305–317.

Yang, V. W.; Zhuang, Z.; Elegir, G. and Jeffrie, T. W. (1995) : Alkaline-active xylanase produced by an alkaliphilic Bacillus sp isolated from kraft pulp J. Ind. Microbiol. 15: 434-441

Yasui, T.; Marui, M.; Kusakabe, I. and Nakanishi, K. (1988) : Xylanases of Streptomyces. Methods Enzymol., 160: 648-654.

Xiong, H., Weymarn, N.; Leisola, M. and Turunen, O. (2003) : Influence of pH on the production of xylanases by Trichoderma reesei Rut C-30. Process Biochem. 39(6): 731-736.

(Received on 7 / 6 / 2009)

الملخص العربى

إنتاج الزيلانيز الخالى من السليوليز من الاستريبتوميسيس بسودوجريسيولاس المعزول محلياً

فتحى عـواد منصـور محمـد السيد عبدالعزيز*

عزة عبدالعزيز مهنى** محمـد فاضـل* أحمد شكرى عبدالرازق*

قسم النبات – كلية العلوم – جامعة المنصورة ، المنصورة

قسم كيمياء الكائنات الدقيقة، المركز القومى للبحوث، الدقى، القاهرة*

قسم النبات، كلية العلوم، جامعة الزقازيق، الزقازيق**

تشكل النفايات النباتية والمخلفات الزراعية (مثل قش الأرز وحطب القطن وغيرها) مشكلة بيئة كبيرة حيث يتم حرق هذه المخلفات، الأمرالذى يؤدى إلى تلوث البيئة والذى يساهم فى تفاقم ظاهرة الاحتباس الحرارى، وقد أجريت كثير من الدراسات التى تهدف إلى التحول الحيوى لتلك المخلفات واستخدامها كأعلاف خشنة غير تقليدية أو فى صناعة تربة البتموس فضلاً عن أهمية إنزيم الزيلانيز فى كثير من الصناعات وبصفة خاصة صناعة الورق، لذا فقد أجريت هذه الدراسة بهدف الحصول على سلالات بكتيرية ذات قدرة على تحلل تلك المخلفات بواسطة قدرتها الإنزيمية الهائلة خاصة إنزيمات الزيلانيز السليوليز، وتناولت هذه الدراسة عزل أجناس مختلفة من بكتريا الأكينوميسيتات من السماد العضوى (البلدى) والتربة من مدينة أجا، هذه العزلات تم إختبارها نوعياً لمعرفة قدرتها على إنتاج الزيلانيز باستخدام ثلاث طرق مختلفة والسلالات التى أظهرت نتائج موجبة تم اختبارها كمياً لاختيار أقواهم فى إنتاج الزيلانيز، وجد من بين هذه العزلات ثلاث سلالات، سلالتين من السماد العضوى (9C,11C) وواحدة من التربة (34S)، لهم قدرة عالية على إنتاج إنزيم الزيلانيز، تم تعريف هذه السلالات بالشكل الظاهرى والطرق الفسيولوچية والكيموتاكسونومية، هذه السلالات هى استريبتوميسيس بسودوجريسيولاس، واستريبتوميسيس ويرينسيز، واستريبتوميسيس جريسيوفلافاس، تم اختيار استريبتوميسيس بسودوجريسيولاس لقدرتها على إنتاج الزيلانيز الخالى من السليوليز، تم تحسين الإنتاج من هذه السلالة بضبط الظروف البيئية والغذائية، الإنزيم الخام أظهر نشاط مثالى عند الأس الهيدروچينى ٦ وأظهر ثبات للأس الهيدروچينى فى المدى من ٥ إلى ١١، هذا الإنزيم أظهر أيضاً درجة حرارة مثلى عند ٦٠ درجة سليزيوس، وله ثبات حرارى يصل إلى ٤٠ درجة سليزيوس، ويحفظ ٦٥٪ من نشاطه عند ٥٠ درجة سليزيوس، ويكبح نشاطه كلياً فيما فوق الـ٦٠درجة سليزيوس.

ISSN 1110-192X JESMUE 5

PRODUCTION OF CELLULASE-FREE XYLANASE FROM A LOCALLY ISOLATED *STREPTOMYCES PSEUDOGRISEOLUS*

Mansour, F. A.[1]; Mohamed S. Abdel-Aziz[2]; Azza, A. AbouZeid[3]; Mohamed, Fadel[2] and Ahmed, S. Abdel-Razik

[1]*Botany Department, Faculty of Science, Mansoura University, Mansoura, Egypt*

[2]*Microbial Chemistry Department, National Research Center, Dokki, Cairo, Egypt*

[3]*Botany Department, Faculty of Science. Zagazig University, Zagazig, Egypt*

Reprint

from

Journal of Environmental Sciences, 2010; Vol. 39

Journal Web Address Jenvsci@mans.edu.eg

THE UNIVERSITY OF MANSOURA - EGYPT