

(cellulase)**SSF**

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<i>A.nadulans</i>	biocout	<i>T.harzianum</i>
(30C° 4 days, pH = 5.2)		
	cellulase	PDA
	()	(SSF)
	<i>T.harzianum</i>	endoglucanase
1.48U/ml <i>A.nadulans</i>		1.54U/ml
		/ (14.8) (15.4)
	exoglucanase	<i>T.harzianum</i>
(0.159U/ml) <i>A.nadulans</i>		0.416U/ml
		/ (1.59) (4.16)
<i>A.nadulans</i>	β -glucosidase	
(5.61U/ml) (6.45U/ml)		<i>T.harzianum</i>
		/ (56.1) (64.5)

Abstract

Two cellulolytic fungi (*T.harzianum*) isolated from the Pesticide (biocout) And (*A.nadulans*) was isolated from a soil of tomato farm, were grew on PDA (4 days ,pH = 5.2 and 30C°),for cellulase production. Cellulase produced using SSF technology by incubation of cotton textile residues (carbon source).

Three type of cellulase were produced :

- Endoglucanase give high activity on the 7th day of incubation for *T.harzianum* (1.54 U/ml) , while *A.nadulans* give at the same day (1.48U/ml) , and they give total activity (15.4 and 14.8/gm carbon source) respectively.

- Exoglucanase give an observed highest activity for *T.harzianum* in the 7th day of incubation (0.416U/ml), while *A.nadulans* have (0.159U/ml) in the same day. Having total activity (4.16 and 1.59/gm carbon source) respectively.
- While β-glucosidase on the other hand , *A.nadulans* give an enzymatic activity higher than *T.harzianum* (6.45 and 5.61) U/ml respectively , and the total activity values for the two fungus are (64.5 and 56.1) / gm carbon source.

B- Exo- 1,4- β-D- glucosidase (Exo- 1,4- β-D- glucose gluconohydrolase)

(E.C.3.2.1.74)

3. β –Glucosidase (β- D-glucoside glucohydrolase) ,(E.C.3.2.1.21)

cellobiase .

¹⁰10

1

(synergism)

5

²(

endoglucanase

exoglucanase

()

3

cellulase

(CBH II)

(CBH I)

exoglucanase

β –Glucosidase

()

1. Endo – 1, 4- β-D-glucanase (Endo – 1, 4- β-D-glucan 4- gluconohydrolase) or CMCCase. (E.C.3.2.1.4)

2. Exo – 1,4- β-D-glucanase

A- Exo-cellobiohydrolase (Exo-1,4-β-D-glucan cellobiohydrolase)

FPAase

(E.C.3.2.1.91).

6

(20\$/Kg) (LSF)

()

¹⁸*S.pulveruletum*

cellulase
endoglucanas(T_{3b}, T_{3a}, T_{2b}, T_{2a}, T₁)

¹⁹ *M. verrucaria*

Trichoderma

T. resei

Aspergillus

14(SCP)

T. harzianum

A. nidulans (biocout)

()

4 PDA
pH=5.2 ° 30
10

¹⁶ *B. Circulans*
¹⁷ *C. Thermocelillum*

(6-5)

° 4

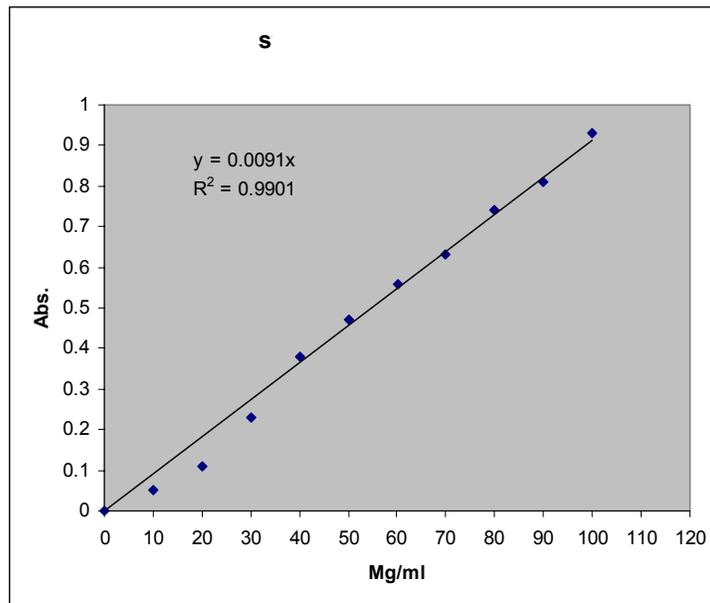
(cellulases)

1

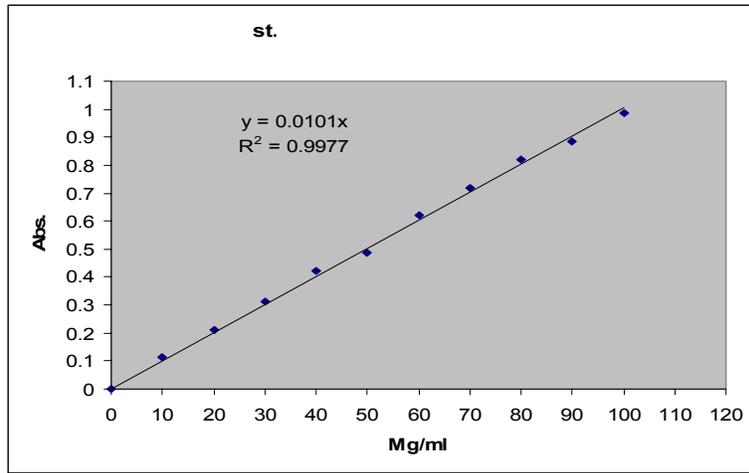
pepton e	CoCl ₂	ZnSO ₄ .7H ₂ O	MnSO ₄ .H ₂ O	FeSO ₄ .7H ₂ O	CaCl ₂	MgSO ₄ .7H ₂ O	Urea	(NH ₄)SO ₄	KH ₂ PO ₄	المادة
0.8	0.02	0.014	0.016	0.05	0.3	0.3	0.3	1.4	2	gm/l

:
 ° 45 (SSF)
 1) 2
 DNS 3 250 ()
²¹(Miller) 20
 10 Autoclave
 10 1 ° 121
 550nm %2
 UV-Visible *A.nadulans T.harzianum*
 spectronic .21D
Exoglucanase(FPase)) ° 30
 - :²⁰ ()
 2 /
 (pH=5.2) 10
 Whatman No.1 :
 (1X6 cm)
 2 20
 1 ° 45 30
 3
 DNS
 (Miller) (whatman No.1)
 10 10 / 3000
 . 550nm :²⁰ **Endoglucanase**
 - :²² **β- Glucosidase** (Na- 4.5
 (p- PNBG 1) CMC)
 nitrophenyl- β-D-glucoside) %0.5
 0.30126) 10Mm .(pH=5.2
 0.5

(10.20.30.40.50.60.70.80.90.100.) μ g/ml 1 100/
 g/ml (pH=5.2)
 30
 1
 3
 ° 45
 (1M) 2
 . DNS
 . 440nm
 (glucose standard curve)
 .1 550nm (100mg/100ml)



(1)
 (10, 20,30,40,50, 60, 70, 80, p- nitro phenol
 90,100) μ g/ml p-nitro
 2 5mM phenol
 1M Na₂CO₃ 2 50mM
 440nm pH 5.2
 2
 . 2



p-nitro phenol (2)

1.54U/ml

A.nadulans

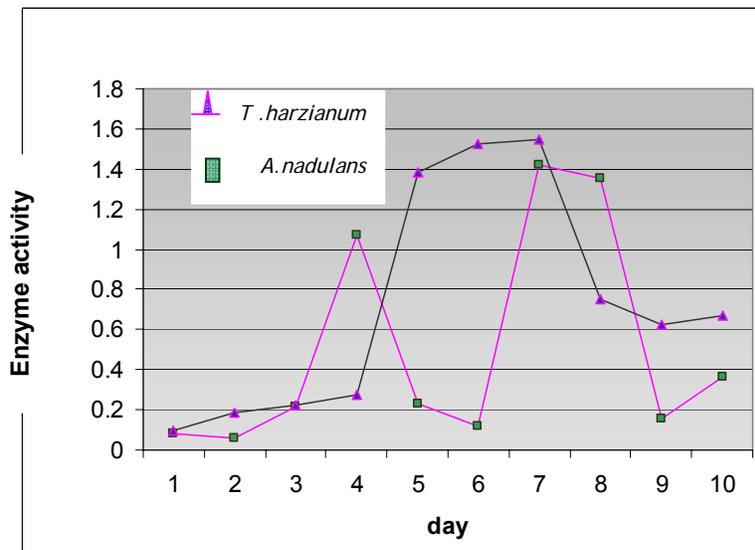
: endoglucanase (CMCase)

(3)

. 1.48U/ml

T.harzianum

endoglucanase(CMCase)



endoglucanase (3)

A.nadulans

/

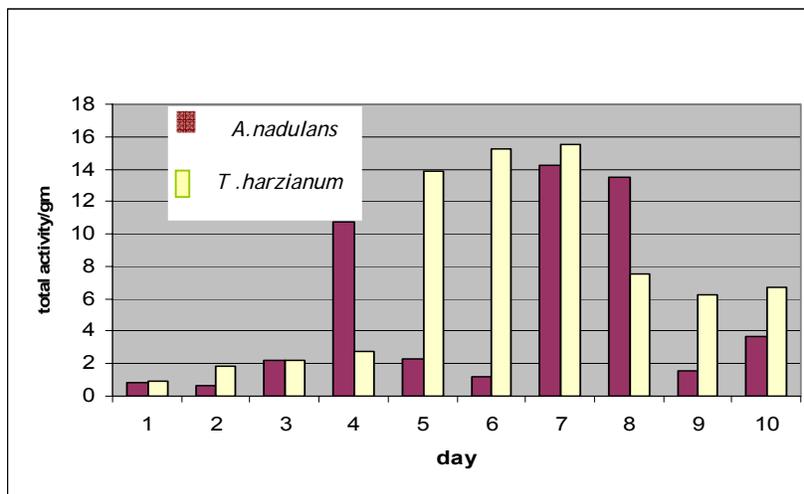
/ 14.8

endoglucanase

(4)

. 15.4

T.harzianum



/endoglucanase (4)

A. niger 3U/ml
 .²⁷ 10gm/l
 0.824U/ml
 .²⁸

Trichoderma
 endoglucanase
 cellulase

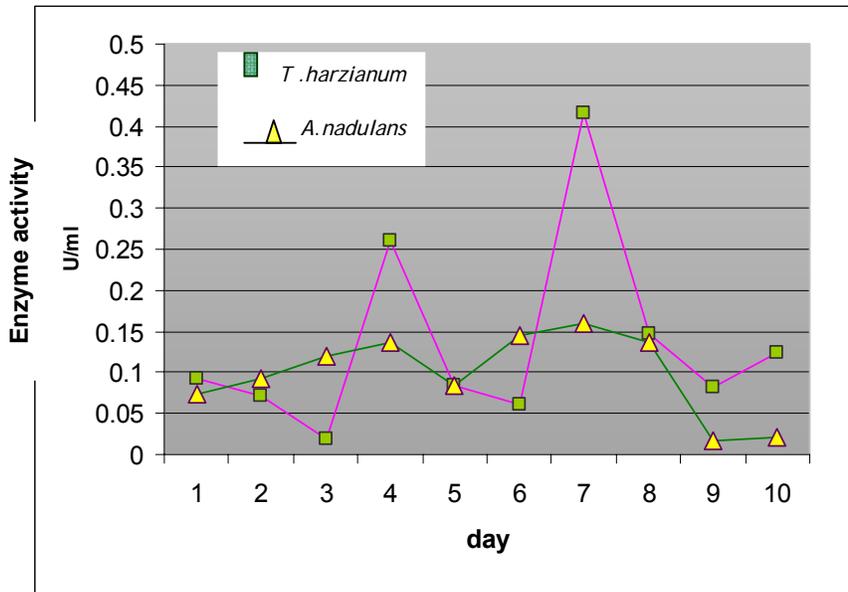
: exoglucanase (FPase)

T. harzianum
 exo-glucanase
 0.416U/ml

Aspergillus
^{23,24,25} *trichoderma*
 cellulase

A. niger
 0.159U/ml

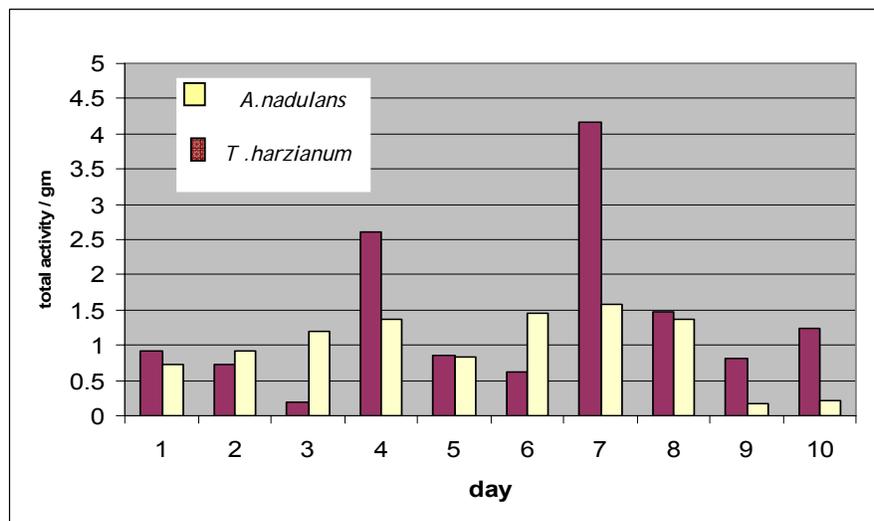
T. reesei
 4.8U/ml
²⁶
T. harzianum



exo-glucanase (5)
(1.59 4.16)

.(6)

A.nadulans *T.harzianum*



/ exo-glucanase (6)

A.fumigatus *A.niger*)

³²(0.340U/ml 0.288U/ml

()

^{29,30}cellulase

Exoglucanases

³⁰ *Bacillus.spp*

.(1.08IU/mg protein)

²⁷*T.harzianum*

³¹ *T.ressei*

0.40U/ml

T.harzianum

: β -glucosidase

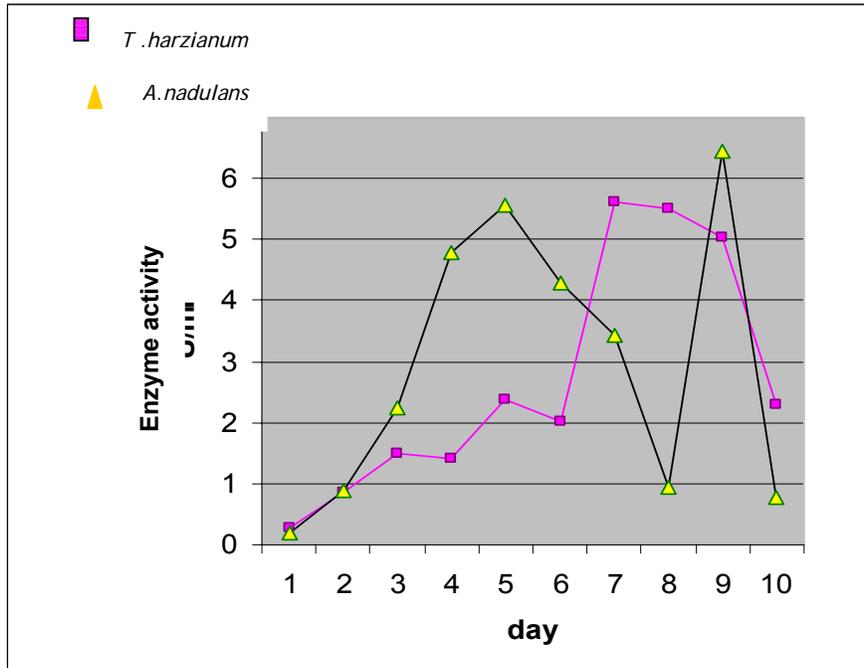
(5)

5.61U/ml

A.nadulans

β -glucosidase

6.45U/ml



β -glucosidase

(7)

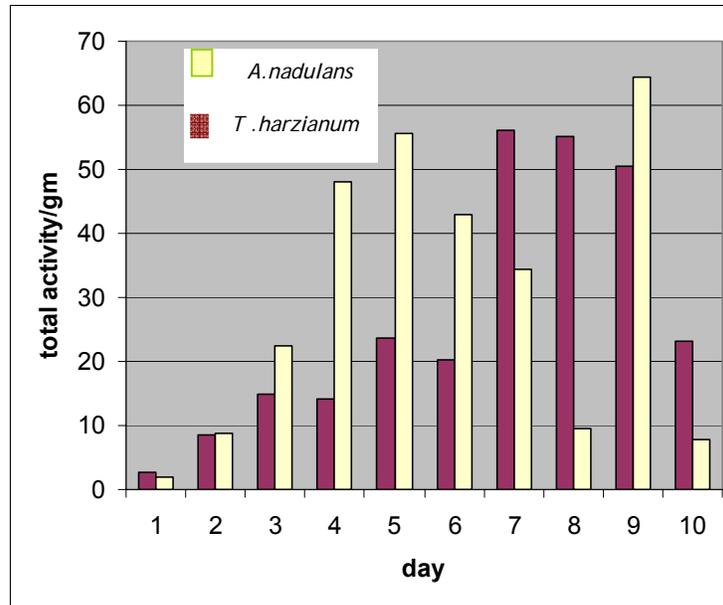
/ 56.1

/ 64.5

A.nadulans

. (endo-exo glucanase)

T.harzianum



/ β -glucosidase (8)

3.07U/ml
. LSF

Aspergillus

*A.nadulans*³⁴ *A.niger*³³
(*A. Terreus*³⁶ *A. oryzae*³⁵
³⁷*trichoderma*

mixed

A.niveus

⁴⁰culture

References :

1. S.chlesinger (*Biogeo chemistry: an analysis of global change*). Academic, San Diego. 1991. p. 443.
2. M.Bhat. *Biotech. Adv.* 2000,**18**: 355.
3. B.Dale. *Biotechnol. Prog.* 1999. **15**: 775
4-R,Mulling, *Enz. Microbiol. technol*,1985,**7**,586.

A.niger

³⁸

sanders

³⁹
²⁸*A.niger*

1.32U/ml

⁴⁰ (*A.niger*

&T.ressei)

- 25- L. Berghem, and L. Pettersson, ,
Eur. J. Biochem 1973, **37**, 21.
- 26 – M. mandals and R. Anderotti,
proc. biochem, 1978, 7.
- 27- S .Rousses, M .Raimbault. *Ann. Microbiol.* 1982. **133B**: 465
- 28- G .Narasimha, A .Sridevi, , M .Subhosh and B. Rajasekhar , *African Journal of Biotechnology* , 2006, **5** (5), 472
- 29- T. OJUMU, B. SOLOMON, E. BETIKU, K. Stephen , and B. AMIGUN, *African Journal of Biotechnology* , 2003, **2** (6), 150
- 30 - X.Júlio, F.Plinho and A. Marco. *Brazilian Journal of Microbiology* 2002, **33**, 213
- 31-M. Zolnorian, M. Hamidi, and R. Yazdanparast, Iran university (abstract).
- 32- . C. Immanuel, M. Akila, P. Iyappa, P. Esakkiraj, and A. Palavesam. *The Internet Journal of Microbiology*. 2007. **3** , 1.
- 33 - H. Yeoh, , T.Tan, S.Chua, and G. Lim. *J. Appl. Microbiol. Biotechnol.* 1988. **4**:425
- 34- P. Bagga, , D.Sandhu, and S.Sharma. *J. Appl. Bacteriol.* 1990. **68**:61
- 35- T. Buachidze, I. Tavobilov, N. Rodionova, and G. Kvesitadze. *Appl. Biochem. Microbiol.* 1987. **23**:187
- 36- C.Riou, J. Salmon, M.Vallier, Z. Gunata, and P. Barre. *Appl. Environ. Microbiol.* 1998. **64**, 3607
- 37 - T. JUHÁSZ , *Food Technol. Biotechnol.* 2003, **41** (1) , 49
- 38- الحسن، أميره محمد علي، " دراسة بعض خواص الإنزيم β -glucosidase المنتجة من الفطر *A.niveus* رسالة ماجستير ، كلية العلوم ، جامعة بابل (1996).
- 39- الحساني ، سعد مدلول ، " دراسة في تشخيص الظروف المثلى لإنتاج الإنزيمات المحللة للسليولوز من الفطريات وأثرها على استهلاك السليولوز وإنتاج البيروتين. رسالة ماجستير . كلية العلوم ، جامعة بابل (2002).
- 40- T. Juhasz , K.kozma, Z. Szengyel and K Réczey, *Food Technol. Biotechnol.* 2003, **41** (1) 40.
- 5- B. Nidetzky, Hayn, M. Macarron, R. and W Steiner. *Biotechnol. Lett.* 1993, **15**, 71.
- 6- T, Teeri, *TIBTEC* 1997, **15**, 160
- 7-R.Tgerdy . *J Sci. Ind. Res.* 1996, **55**, 313
- 8- A. Archana, and T. Satyanarayana, *Enzyme Microb. Technol.*, 1997, **21**, 12
- 9.H.Klozlawaski, A. Trozynska and T. Sokoski . *cielat .Przem.spoz.*, 1976, **30**, 311
- 10- T. Tzanov, J. Andreaus, G. Guebitz, and A. Paulo, *Electronic J. Biotech.* 2003, **6**, 3.
- 11- K. Kleman, M. Aho, T. Teeri, and T. Kirk. *Appl & Environ Microbiol.* 1996, **62** (8) 2883.
- 12- K.Singh, A K. Puniya and K.Singh, *J.Sci & Indst. Res.*, 1996, **55**(5), 472.
- 13-A.Noon, *Aric.wastes*, 1986, **17**(3), 229.
- 14- دلالي ، باسل كامل " موضوعات مختارة في التكنولوجيا الحيوية (1993).
- 15 – A. Pandey, , *in Solid State Fermentation* , Wiley Eastern Publishers, New Delhi, 1994. 3.
- 16- O.Thikhomirov, V.Stobve, and A.Klysov . *Prikl.biokhem.microbiol.* 1989. **25**(1), 48.
- 17-G.Halliwell , and N.Halliwell , *Biochem.Biopys.Acta*, 1989. **922**(2), 22.
- 18- K.Almin, K. Eriksson, and N. Pettersson, *Eur.J.Biochem* , 1975, **51**, 207.
- 19- L.Trived, and K.Rao . *Ind. J. Exp. Biol.* 1980. **18** (3), 240
- 20-M Mandels, R.Andreotii and C.Roche *Biotechnol. Bioeng. Symp.* (1976) **6**, 21
- 21-G.Miller. *Anal.chem.*, 1959, **31**, 426.
- 22-C.Kubick *Eur.J.Appl .Microbiol. Biotechnol.* 1981, **13**, 226.
- 23-Miettinen-Oinonen, Arja.. *VTT Publications Espoo* 2004, 550. 96
- 24- M. Boat, and S. Bhat. *Biotechnol. Adv.* 1997. **15**, 583. 620.