J. Asiat. Soc. Bangladesh, Sci. 39(1): 43-51, June 2013

OPTIMIZATION OF CULTURE CONDITIONS FOR THE PRODUCTION OF XYLANASE BY TWO THERMOPHILIC FUNGI UNDER SOLID STATE FERMENTATION

KUMKUM AZAD[1], MD. ABDUL HALIM AND FEROZA HOSSAIN
Department of Botany, Jahangirnagar University, Savar, Dhaka-1342, Bangladesh

Abstract

Two thermophilic fungi, *Thermomyces lanuginosus* BPJ-10 and *Rhizomucor pusillus* BPJ-2 were studied under solid state fermentation (SSF) using wheat bran for the production of thermostable xylanase. The optimum time required for the production of xylanase was found to be 4 days and 7 days for *R. pusillus* BPJ-2 and *T. lanuginosus* BPJ-10 respectively. The optimum temperatures for the production of xylanase by *R. pusillus* BPJ-2 and *T. lanuginosus* BPJ-10 were 45°C and 50°C respectively. The maximum activity of xylanase (1.685 IU/ml and 0.075 IU/ml) was exhibited by *T. lanuginosus* BPJ-10 and *R. pusillus* BPJ-2 at pH 7.0 and pH 4.0 respectively. The optimum moisture content for maximum xylanase production was 90% for both fungi.

Key words: Xylanase, Thermophilic fungi, *Thermomyces lanuginosus* and *Rhizomucor pusillus*

Introduction

Microbial technology is applied to produce xylanolytic enzymes for improving the quality of inferior lignocellulosics using thermophilic fungi under solid state fermentation. Although most of the sources of xylanase are reported to be produced by mesophilic fungi (Gattinger *et al*. 1990 and Yang *et al*. 2006), one of the major problems encountered in the utilization of these enzymes is their poor temperature stability and low hydrolysis rate of the lignocellulosics (Akhtar *et al*. 2006).

Thermophilic fungi carry out enzymatic hydrolysis at elevated temperature over prolonged period of time due to their inherent superior thermostability (Akhtar *et al*. 2006 and Yang *et al*. 2006). Moreover, thermostable enzymes can be recovered and purified at ambient temperature. Diffusion and other chemical processes of thermostable enzymes are accelerated at high temperature resulting increased reaction rate. On the otherhand, large scale fermentation with thermophiles is technically and economically more feasible than mesophilic counterparts (Khan *et al*. 1996).

Thermostable lignocellulolytic enzymes such as xylanase has significant potential applications in biodegradation of various industries including chemicals, fuel, food, brewery and wine, animal feed, fiber, textile and laundry, pulp and paper and agriculture (Ghatora *et al*. 2006, Howard *et al*. 2003, Senthilkumar *et al*. 2005 and Virupakshi *et al*. 2005). In the present study, an attempt has been made to investigate two thermophilic

[1] Corresponding author: E-mail: azadmow@yahoo.com

fungi, *Thermomyces lanuginosus* BPJ-10 and *Rhizomucor pusillus* BPJ-2 for the production of thermostable xylanase to optimize some of the cultural parameters such as temperature, pH, incubation time and moisture content under solid state fermentation for maximization of the production of enzymes.

Materials and Methods

Two thermophilic fungi, *Thermomyces lanuginosus* BPJ-10 and *Rhizomucor pusillus* BPJ-2 were isolated and identified at the laboratory of Plant Physiology and Biochemistry, Department of Botany, Jahangirnagar University, Savar, Dhaka. The fungi were cultured on PDA media and maintained at 50°C and 45°C temperature for *T. lanuginosus* BPJ-10 and *R. pusillus* BPJ-2 respectively. The cultures were incubated for five days and the spores of the cultures were washed with sterile water and the resulting suspention (2.5×10^5 spores per ml) was used as inocula. These fungi were employed for the production of xylanase under solid state fermentation described by Halim *et al*. (2001) and Mohiuddin (1992). To optimise the enzyme production following cultural conditions were investigated by using wheat bran as a solid substrate. Wheat bran (10g) was taken in each of the 250 ml conical flask. The substrates were mixed thoroughly with distilled water for maintaining the suitable amount of moisture content. The flasks were then properly cotton plugged and autoclaved at 121°C for 15 minutes. Each flask was inoculated with 2 ml of spore suspension. To find the effect of temperature for maximization of enzyme production, inoculated flasks were incubated at temperature ranging from 35° to 60°C. To investigate the effect of incubation period for maximization of enzyme production, the inoculated flasks were incubated for 10 days at 45°C and 50°C for *T. lanuginosus* BPJ-10 and *R. pusillus* BPJ-2 respectively. Enzyme was extracted and assayed at every 24 hours starting from the 1st day upto 10th day of inoculation. For the determination of the effect of moisture content on enzyme production 50, 60, 70, 80, 90 and 100% distilled water were added with solid substrate. The pH of the initial culture was adjusted to 4, 5, 6, 7, 8 and 9 for both fungi to find its optimum value.

After optimum incubation time (7 days for *T. lanuginosus* BPJ-10 and 4 days for *R. pusillus* BPJ-2) 100 ml of 1% NaCl solution was added to the culture and mixed properly to each fermented biomass. In order to disperse the mycelia bound biomass, the flasks with moldy substrate was shaken in a shaker for 45 minutes at 150 rpm. The fermented slurry was first filtered with nylon cloth and then extracts were filtered with Millipore membrane filter paper (0.25μ). Clear filtrate was obtained and centrifuged at 4500 rpm for 15 minutes. The clear supernatants were used for the determination of enzyme activity. The amount of reducing sugar was estimated by Dinitro salicylic acid (DNS) method (Millar 1959). Xylanase activitiy was assayed by standard procedure of Mohiuddin (1992). The enzyme activity was calculated and expressed in International Unit (IU). One IU is the amount of enzyme which liberates 1 μmol of reducing sugar per minute under assay conditions. The enzyme activities were measured from average of

three replicates for each parameter. The relative percentage of xylanase activity was calculated by taking the maximum activity as 100%.

Results and Discussion

The optimum time required for xylanase production was 4 days and 7 days for *R. pusillus* BPJ-2 and *T. lanuginosus* BPJ-10 respectively. The maximum activities of xylanase for *T. lanuginosus* BPJ-10 was 1.49 IU/ml, whereas the maximum activities of xylanase for *R. pusillus* BPJ-2 was 0.085 IU/ml (Table 1). The activity of xylanase was reached at the peak on 7th day of incubation by *T. lanuginosus* BPJ-10 and on 4th day of incubation by *R. pusillus* BPJ-2. Then it declined sharply for both fungi (Fig.1). Akhtar *et al*. (2006) and Rashid *et al*. (2005) also reported that the optimum time required for the maximum production of xylanase was 7 days for *T. lanuginosus*.

Table 1. Effect of incubation period on production of xylanase by *T. lanuginosus* BPJ-10 and *R. pusillus* BPJ-2.

Incubation period (days)	Name of the fungi			
	T. lanuginosus BPJ-10		*R. pusillus* BPJ-2	
	Xylose conc. (mg/ml)	Xylanase activity (IU/ml)	Xylose conc. (mg/ml)	Xylanase activity (IU/ml)
1th	1.6	0.085	0.2	0.004
2th	9.2	0.49	1.76	0.031
3th	18.6	0.992	3.2	0.057
4th	21.8	1.163	4.76	0.085
5th	24.2	1.29	2.56	0.046
6th	26.2	1.397	1.28	0.023
7th	28	1.49	0.76	0.014
8th	13.4	0.715	0.56	0.01
9th	11	0.587	0.28	0.005
10th	6.8	0.363	0.2	0.004

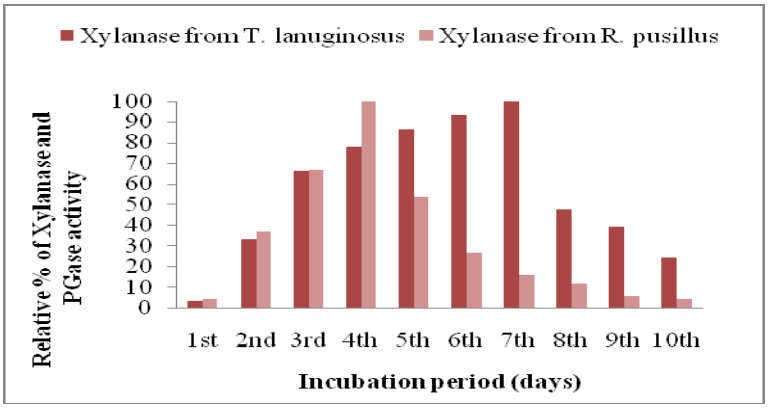

Fig.1. Relative % of xylanase activity by *T. lanuginosus* BPJ-10 and *R. pusillus* BPJ-2 in different incubation periods.

Moisture content in solid substrate is an important controlling factor for enzyme production in solid state fermentation (Nagai and Nishio 1980). An increase in moisture content adversely affects the enzyme production. This is probably due to poor diffusion of oxygen and release of toxic metabolites. The higher percentage of moisture content might be required at high temperature. After 4 to 5 days of incubation moisture content decreased in the substrate due to utilization of moisture by fungi. The problem of drying out of the medium can be overcome by maintaining a constant relative humidity at 50-60% in the incubator. In case of *T. lanuginosus* BPJ-10, xylanase exhibited the maximum activity (1.013 IU/ml) at 90% moisture content (Table 2). On the other hand, Akhtar *et al*. (2006) observed that 80% moisture content was congenial to the growth of the fungus and xylanse production by *T. lanuginosus*. Another fungus *R. pusillus* BPJ-2 also exhibited xylanase activity (0.078 IU/ml) at 90% moisture content (Table 2). The relative percentage of xylanase activity was calculated by taking the maximum activity as 100%. It increased gradually from 50% to 90% moisture content and then started to drop the trend of increase for both fungi (Fig. 2).

Table 2. Effect of moisture content on production of xylanase by *T. lanuginosus* BPJ-10 and *R. pusillus* BPJ-2.

Different Moisture Content (%)	Name of the fungi			
	T. lanuginosus BPJ-10		*R. pusillus* BPJ-2	
	Xylose conc. (mg/ml)	Xylanase activity (IU/ml)	Xylose conc. (mg/ml)	Xylanase activity (IU/ml)
50	1.4	0.074	1.28	0.023
60	12	0.64	2.24	0.040
70	15	0.8	3.12	0.055
80	16.8	0.896	3.72	0.066
90	19	1.013	5.52	0.078
100	15.8	0.843	2.56	0.045

Average of three replicates.

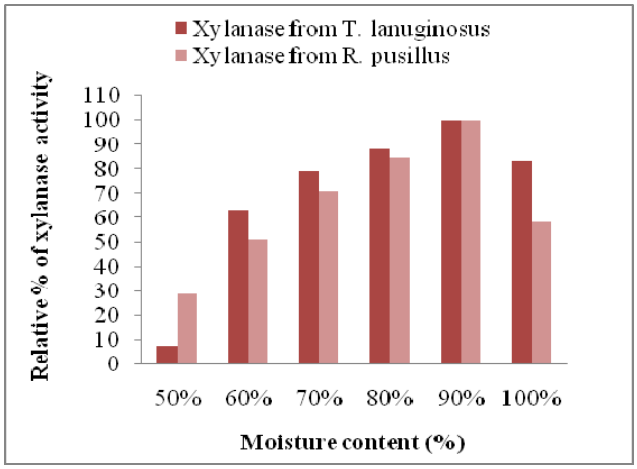

Fig. 2. Relative % of xylanase activity by *T. lanuginosus* BPJ-10 and *R. pusillus* BPJ-2 in different moisture levels.

The fungi were grown at different temperature ranging from 40 to 60°C to find out the optimum temperature for growth and maximum enzyme production. The optimum temperature for the maximum production of xylanase (1.568 IU/ml) was 50°C for *T. lanuginosus* BPJ-10 and the optimum temperature for the maximum production of xylanase (0.089 IU/ml) was 45°C for *R. pusillus* BPJ-2 (Table 3). The relative percentage of xylanase activity was calculated by taking the maximum activity as 100% and it was exhibited at 50ºC and 45ºC temperature for *T. lanuginosus* BPJ-10 and *R. pusillus* BPJ-2 respectively (Fig. 3).

Table 3. Effect of different temperature on production of xylanase by *T. lanuginosus* BPJ-10 and *R. pusillus* BPJ-2.

Temperature (ºC)	Name of the fungi			
	T. lanuginosus BPJ-10		*R. pusillus* BPJ-2	
	Xylose conc. (mg/ml)	Xylanase activity (IU/ml)	Xylose conc. (mg/ml)	Xylanase activity (IU/ml)
35	8	0.426	2.4	0.043
40	25	1.333	4.24	0.075
45	27	1.44	5.04	0.089
50	29.4	1.568	3.84	0.068
55	24	1.28	1.4	0.025
60	6	0.32	0.28	0.005

Average of three replicates.

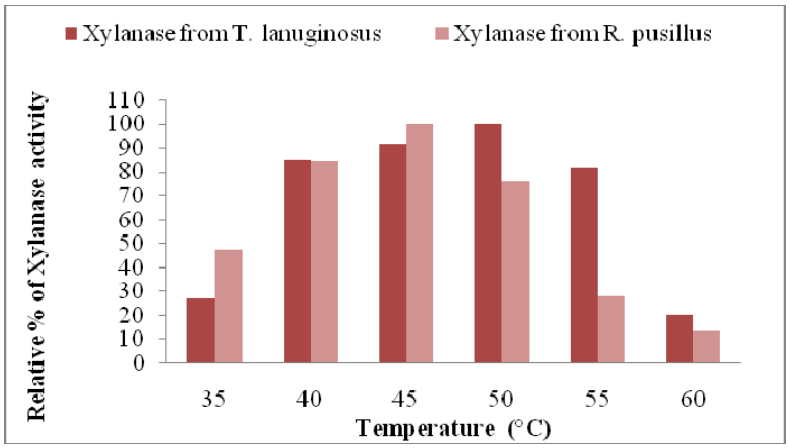

Fig. 3. Relative % of xylanase activity by *T. lanuginosus* BPJ-10 and *R. pusillus* BPJ-2 in different temperatures.

Reports from many workers revealed that maximum thermophilic fungi and other microorganisms showed their higher performance between 50 to 55°C temperatures (Debsarma 1989, Bodine and Stutzenberger 1992 and Nowab 1992) which corroborates with the result of the present study. According to Akhtar *et al.* (2006) the optimum temperature found for the maximum production of xylanase by *T. lanuginosus* was 55°C.

Initial pH of culture medium played an important role on the production of enzyme. Thermophilic fungi grew satisfactorily in a minimal medium if the pH of the medium was controlled between 5.5 and 7.0 (Maheshwari *et al.*2000). *T. lanuginosus* BPJ-10 and *R. pusillus* BPJ-2 exhibited good performance in pH range of 4 to 8. However, the maximum activity of xylanase (1.685 IU/ml) was exhibited at pH 7.0 in case of *T. lanuginosus* BPJ-10 (Table 4). On the other hand, the maximum activity of xylanase (0.075 IU/ml) was exhibited at pH 4.0 in case of *R. pusillus* BPJ-2 (Table 4). The percentage of xylanase activity was calculated by taking the maximum activity as 100% and it was found at pH 7 and pH 4 for *T. lanuginosus* BPJ-10 and *R. pusillus* BPJ-2 respectively (Fig. 4). Akhtar *et al*. (2006) reported that the highest activity of xylanase from *T. lanuginosus* was at pH 6.5. According to Gomes *et al*. (1993) the required pH for the maximum production of xylanase by *T. lanuginosus* lies between 7 and 7.5 and Nawab (1992) found it between 6.0 and 7.25. The results of the present study agree well with those findings.

Table 4. Effect of different level of pH on the production of xylanase by *T. lanuginosus* BPJ-10 and *R. pusillus* BPJ-2.

Different level of pH	Name of the fungi			
	T. lanuginosus BPJ-10		*R. pusillus* BPJ-2	
	Xylose conc. (mg/ml)	Xylanase activity (IU/ml)	Xylose conc. (mg/ml)	Xylanase activity (IU/ml)
pH 4	20.6	1.099	4.2	0.075
pH 5	24.2	1.290	3.64	0.069
pH 6	30	1.6	3.36	0.059
pH 7	31.6	1.685	1.88	0.033
pH 8	24.2	1.290	1.44	0.026
pH 10	18.4	0.981	0.56	0.001

Average of three replicates.

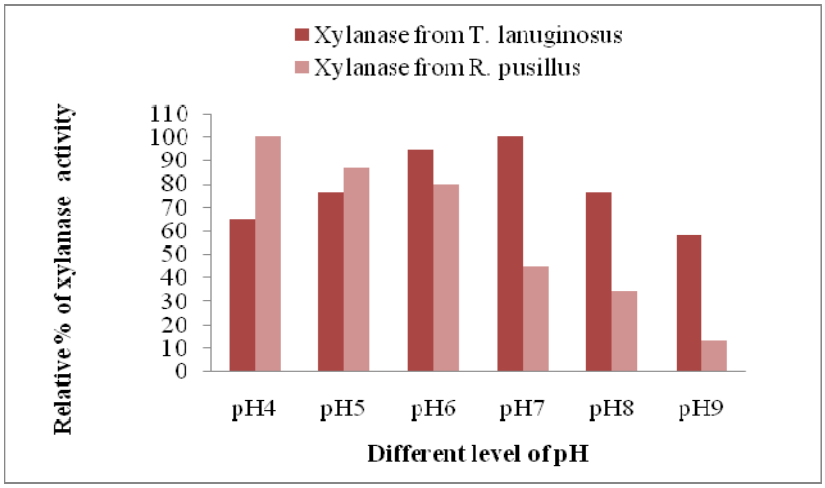

Fig.4. Relative % of xylanase activity by *T. lanuginosus* BPJ-10 and *R. pusillus* BPJ-2 at different pH.

It may be concluded from the present findings that the thermophilic fungi, *T. lanuginosus* BPJ-10 and *R. pusillus* BPJ-2 have potential capability of producing xylanase under the physiological conditions and the cultural environment described above. Though both fungi exhibited maximum activity of xylanase in the same level of moisture content (90%), their level of temperature and pH were different. Moreover, *R. pusillus* BPJ-2 exhibited maximum activity of xylanase on 4th day of incubation whereas it was exhibited by *T. lanuginosus* BPJ-10 on 7th day of incubation, which reveals that *R. pusillus* BPJ-2 is more economical than that of *T. lanuginosus* BPJ-10 in the respect of time duration. Besides these, *T. lanuginosus* BPJ-10 is more potential thermophilic fungi

than *R. pusillus* BPJ-2 with regard to xylanase producing capacity in solid state fermentation.

References

Akhtar, N., F. Hossain and G. Mohiuddin. 2006. Optimization of cultural parameters under solid-state fermentation for the production of Xylanase by *Thermomyces Lanuginosus*. *Bangladesh J. Life Sci.* **18**(1): 85-90.

Bodine, A. B. and R. J. A. Stutzenberger. 1992. Xylanase production by *Thermomonospora curvata. J. Appl. Bacteriol*. **72**: 504-511.

Debsarma, G. D. 1989. Biochemical investigations on jute retting. *Ind. J. Agri. Sci*.**19**: 453-458.

Gattinger, L. D., Z. Duvnjak and A. W. Khan. 1990. The use of canola meal as a substrate for xylanase production by *Trichoderma reesei. Appl. Microbiol. Biotechnol*. **33**: 21-25.

Ghatora, S. K., B. S. Chadha, A. K. Badhan, S. H. Saini and M. K. Bhat. 2006. Xylanases from Fungi. *BioResourses*. **1**(1): 18-33.

Gomes, J., I. Gomes, H. Esterbauer, M. Sinner and W. Steiner. 1993. Production of high level of cellulose free and thermostable xylanase by a wild strain of *Thermomyces lanuginosus* in laboratory and pilot scales using lignocellulosic masterials. *Appl. Microbial. Biotechnol*. **39**: 700-709.

Halim, M. A., F. Hossain, A. S. K. Hasib and G. Mohiuddin. 2001. Optimization of cultural condition under solid-state fermentation for the production of cellulase, xylanase and pectinase by *Phaenerochaete Chrysosporium* DSM 6909. *J. Asiat. Soc. Bangladesh, Sci.* **27**(2): 195-203.

Howard R. L., E. Abotsi, E. L. Jansen van Rensburg and S. Howard. 2003. Lignocellulose biotechnology: issues of bioconversion and enzyme production. *Afr. J. Biotechnol.* **2** (12): 602-619.

Khan, S. N., D. J. Gomes and N. Choudhury. 1996. Optimization of cultural medium for the production of xylanase from *Thermomyces lanuginosus* TF5 using Box-Wilson method. *Bangladesh J. Life Sci*. **8** (2): 35-44.

Maheshwari, R., G. M. K. Bharadwaj and Bhat. 2000. Thermophilic Fungi: Their Physiology and Enzymes. *Microbiology and Molecular Biology Reviews*. **64** (3): 461-488.

Miller, L. G. 1959. Use of dinitrosalicylic acid reagent for determination of reducing sugars. *Analytical Chemistry.* **31**: 426-428.

Mohiuddin, G. 1992. *A Manual for imporved processing technique for low grade jute and cuttings*. pp. 6-19.

Nagi, S. and N. Nisho.1980. Proceeding of the second syposium on bioconversion and biochemical engineering. Ghose. T.K. (Eds.). New Delhi**. 1**: 399.

Nawab. 1992. Production, optimization and partial characterization of xylanase from *Thermomyces lanuginosus*. M.Sc. thesis, Dept. of Microbiology, Dhaka University. Pp. 40-45.

Rashid, M., Z. U. M. Khan, S. K. A. Hasib and G. Mohiuddin. 2005. Studies of pectinase production potential of *Thermomyces lanuginosus*-M3 and its effect on the improvement of jute fibre. *Bangladesh J. Life Sci.* **17**(1): 63-67.

Senthilkumar, S. R., B. Ashokkumar, K. Chandra Raj and P. Gunasekaran. 2005. Optimization of medium composition for alkali-stable xylanase production by *Aspergillus fischeri* Fxn 1 in solid-state fermentation using central composite rotary design. *Bioresour. Technol.* **96**: 1380– 1386.

Virupakshi, S., K. G. Babu, S. R. Gaikwad and G. R. Naik. 2005. Production of a xylanolytic enzyme by a thermoalkaliphilic *Bacillus* sp. JB-99 in solid state fermentation. *Process Biochem*. **40**: 431–435.

Yang, S. Q., Q. J. Yan, Z. Q. Jiang, L. T. Li, H. M. Tian and Y. Z. Wang. 2006. High-level of xylanase production by the thermophilic *Paecilomyces themophila* J18 on wheat straw in solid-state fermentation. *Bioresource Technology*. **97**: 1794–1800.

(Received revised manuscript on 22 April 2013)