



EFFICIENT PRODUCTION OF XYLANASE ENZYME BY FUNGAL SPECIES USING LOW COST AGRO-INDUSTRIAL SUBSTRATES

Botany

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ABSTRACT

Xylanase enzyme production has been reported in several microorganisms including bacteria, fungi and actinomycetes. Filamentous fungi are unique for extracellular enzyme production and ease of cultivation. Fungal isolates were obtained from decomposing saw dust and screened for their abilities for producing xylanase enzyme by using Remazole Brilliant Blue R-D-xylan dye diffusion method. *Aspergillus niger*, *Aspergillus terreus*, *Chrysosporium* sp., *Fusarium moniliforme* and *Penicillium* sp. showed positive results. These fungal strains were employed further for production of xylanase enzyme. Submerged fermentation was carried out under varying pH of culture media and different agro-industrial substrates as carbon source. *Aspergillus niger* and *Aspergillus terreus* showed maximum xylanase enzyme production at pH5 (204.686 U/mL and 320.985 U/mL respectively). *Aspergillus terreus* produced maximum xylanase (2095.71 U/mL) when sugarcane bagasse was used as a sole carbon source in the culture medium.

KEYWORDS

agro-industrial, submerged fermentation, sugarcane bagasse, xylanase

INTRODUCTION

Xylan the major component of hemicellulose present in plants accounts for 20- 35% of the total tropical plant biomass [5, 8]. Xylans consists of backbone of β -D-1, 4-linked xylopyranoside units substituted with acetyl, glucuronosyl, and arabinosyl side chains [11]. The breakdown of xylans to xylose and xylo-oligosaccharides is facilitated by xylanases. Xylanase (E.C 3.2.1.8) are a group of five enzymes that degrades β -1, 4 xylan by cleaving β -1,4glycosidic linkages randomly to form the products [24]. Due to their industrial and biotechnological applications xylanases have gained widespread importance [21]. They are used in the manufacturing of bread, food and drinks, clarification of juices, improving the nutritional properties of agricultural silage and grain feed, textile industries, paper pulp and bio-bleaching industries, pharmaceutical and chemical applications [12, 18]. Several microorganisms such as bacteria, fungi and actinomycetes have been reported to be the sources for xylanase enzyme [8]. Filamentous fungi are unique and more preferred due to their capabilities for extracellular enzyme production and higher enzyme level than those of yeast and bacteria [22, 23]. White rot basidiomycetes like *Phanerochaete chrysosporium* and *Coriolus versicolor* also produce xylanolytic enzymes [1]. Species of *Trichoderma*, *Aspergillus*, *Penicillium* etc. have been utilized for the production of commercial xylanases [12, 2]. Industrial applications require large scale enzyme production and hence involve high cost. Therefore, the use of agro-industrial residues as low- cost substrates for enzyme production by fungal strains can be a significant way for reducing the production cost [15].

The present study was carried out to screen highly potent xylanase enzyme producers at their optimum pH and using cheap lignocellulosic substrates.

MATERIALS AND METHODS:

1. Isolation of fungi:

Fungi were isolated from decomposing saw dust using a tenfold serial dilution-plating technique on potato dextrose agar (PDA) plates. The plates were incubated at $28^{\circ} \pm 2^{\circ}$ C. The pure cultures were then transferred to PDA slants and maintained at 4°C and sub-cultured till further use.

2. Identification of Fungi

The isolated fungi were identified by observing their macroscopic characters such as colour, appearance and diameter of colonies and microscopic characteristics [4] and the results were confirmed from Agharkar Research Institute, Pune, Maharashtra, India.

3. Qualitative screening for xylanolytic activity:

The fungal isolates were screened for their abilities to produce extracellular xylanase enzyme using Remazole Brilliant Blue R-D-xylan dye diffusion method with slight modifications [17]. 10 ml of xylanolysis basal medium supplemented with 1.6% w/v agar were autoclaved in test tubes and allowed to solidify. To these tubes, 0.2 ml

of separately autoclaved and slightly cooled basal medium that was supplemented with 0.5 % RBB-xylan was added as an overlay and then allowed to solidify. These tubes were then inoculated with the fungal isolates and incubated at 27° C and examined daily for 15 days for the migration of the dye.. The uninoculated tube was retained as Control.

4. Preparation of fungal inoculums for submerged fermentation:

The fungal isolates showing comparatively more migration of the RBB-xylan agar dye were selected for further studies. Four mycelial plugs (8mm diameter) were made on 7 day old culture PDA plate and the mycelial mats on the plugs were carefully scraped so as to remove agar and aseptically added to the flasks containing 10ml of Sabouraud's dextrose under aseptic conditions. The flasks were incubated at $28^{\circ} \pm 2^{\circ}$ C on an orbital shaker at 150 rpm for 48 hrs. to obtain large quantity of active mycelia.

5. Preparation of substrate:

The agro- industrial substrates like sugarcane bagasse, mixed sawdust and coconut fruits were procured from the local market, Thane. These lignocellulosic substrates were cut into pieces and repeatedly washed with distilled water, sundried and grinded into powder. The powders were then sieved, autoclaved and stored in a sterile airtight container till further use.

6. Submerged Fermentation

Mandel and Weber's medium [13] was used for the production of enzyme by submerged fermentation which was modified by adding Tween 80 0.1% v/v and beech wood xylan 1% as carbon source.

25 ml of the media was dispensed into 250 ml of Erlenmeyer flask and sterilized at 121°C for 15mins. The flasks were then inoculated with 5ml of fungal inoculums and then incubated for 6 days at 28°C on rotary shaker at 150 rpm. The contents of each flask were filtered through Whatman's filter paper no.1. The filtrate was centrifuged at 5000 rpm for 20 minutes and the supernatant was used as crude enzyme extract. The experiments were performed in triplicates.

7. Measurement of Xylanase activity:

Xylanase activity was determined by measuring the release of reducing sugars from xylan with the DNSA method [14]. The reaction mixture tube containing 0.5ml of 1 % beech wood xylan in 0.5M sodium citrate buffer pH 4.8 and 0.5 ml crude enzyme extract was incubated at 50° C for 30 minutes [3]. The reaction was stopped by addition of 3ml of DNSA and then kept in boiling water bath for 5 mins and then cooled [14]. The colour developed was read at 540 nm on spectrophotometer. Xylanase activity was expressed as the amount of enzyme that releases 1 μ mol of reducing sugar (xylose equivalent) per minute per milliliter under the assay conditions.

8. Optimization of culture conditions for xylanolytic enzymatic activity

a. Effect of pH

The culture flasks containing 50 ml of the culture medium were inoculated with the active mycelia. The range of pH of the culture medium used for xylanase enzyme production was 4,5,6,7 and 8.

b. Effect of carbon sources (agro-industrial wastes) on enzyme production:

Submerged fermentation of the selected fungal strains was carried out by using various lignocellulosic substrates like sugarcane bagasse, fibrous mesocarp of coconut and saw dust as carbon sources. 1% w/v of carbon sources were then added to the fermentation media replacing xylan.

RESULTS AND DISCUSSION

1. Isolation and identification of fungi:

Seven different fungal species were isolated and identified as *Aspergillus niger*, *Aspergillus terreus*, *Chrysosporium sp.*, *Fusarium moniliforme*, *Paecilomyces sp.*, *Paecilomyces variotii* and *Penicillium sp.*

2. Qualitative screening:

The screening of the fungal isolates for their xylanase producing capabilities was studied by Xylan-agar dye diffusion method [17]. The diffusion of the dye to the lower clear zone indicated the xylanolytic activity of the fungi was observed in the tubes inoculated with the fungal isolates with varying degrees (Fig.1). *Aspergillus niger* and *A. terreus* showed high degree of migration of dye; *Chrysosporium sp.*, *Fusarium moniliforme* and *Penicillium sp.*, showed moderate migration whereas *Paecilomyces sp.*, *Paecilomyces variotii* showed very less migration of the dye.

3. Xylanase enzyme production:

The comparative study for xylanase production by submerged fermentation was carried out using *Aspergillus niger*, *A. terreus*, *Chrysosporium sp.*, *Fusarium moniliforme* and *Penicillium sp.*

a. Effect of pH

The pH of the culture medium greatly influences the growth of the microorganisms as well as its enzyme production. Xylanase enzyme production by fungal isolates at different pH range (4-8) was studied. The highest activity was reported by *Aspergillus terreus* at initial pH 5.0 (320.985U/mL), followed by *A. niger* (204.686 U/mL) at pH 6.0. Optimum pH for *Chrysosporium sp.* and *Penicillium sp.* was found to be 4 and for *Fusarium moniliforme*, it was pH 6 (166.889 U/mL) (Table 1). The endoxylanases show high activity between pH 4.0 and pH 6.5, but optimal conditions have been found outside these ranges also [18]. Various studies have reported acidic pH (5-6.5) as most appropriate for maximum production of enzyme from fungi [7]. Knob *et al.* [10] has reported *Penicillium glabrum* highly potent in the range of 4.5 to 6.5.

b. Effect of carbon sources

As shown in the Table 2, sugarcane bagasse as a carbon source was found to be the best inducer for xylanase production. *Aspergillus terreus* produced maximum xylanase (2095.71 IU/mL) which was almost double than that of *Penicillium sp.* (1062.393 IU/mL). Various lignocellulosic materials like wheat bran, corn cobs, paddy straw, cocoa husk etc. have been reported to be efficient sources for production of xylanase. Wheat bran has been reported as a promising carbon source [9, 16]. Studies on xylanase production by few *Aspergillus* species have been carried out [20]. Xylanase production in shake flasks was carried out by using the mineral salt medium supplemented with 1% w/v birch wood xylan. *Aspergillus niger* MS80 and *A. flavus* MS 172 showed highest and similar xylanase activity (1.243IU/mL) and the least was observed in *Aspergillus terreus* MS 105(0.317 IU/ml). This difference in xylanase production may be due to the variable nature and degradation of the hemicellulosic material [19].

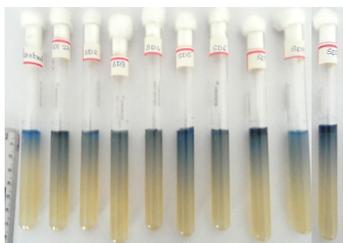


Fig.1 : Remazole Brilliant Blue-dye degradation by fungal isolates from decomposing saw dust

SD1: *Aspergillus niger*; SD6: *Aspergillus terreus*;
SD2 & SD3: *Paecilomyces variotii*; SD7: *Paecilomyces sp.*;
SD4: *Penicillium sp.*; SD8: *Chrysosporium sp.*;
SD5: *Aspergillus niger*; SD9: *Fusarium moniliforme*

Sr. No.	Fungi	4	5	6	7	8
1	<i>Aspergillus niger</i>	151.189	204.68**	172.122	102.92	120.36
2	<i>Aspergillus terreus</i>	290.748	320.98***	279.118	265.16	190.14
3	<i>Chrysosporium sp.</i>	179.682	148.863	125.021	100.59	77.920
4	<i>Fusarium moniliforme</i>	131.418	154.678	166.889	120.36	88.968
5	<i>Penicillium sp.</i>	65.709	95.946	102.924	123.27	93.620

Sr. No.	Fungi	Sugarcane bagasse	Saw dust	Coconut coir
2	<i>Aspergillus niger</i>	1650.27	479.152	1109.494
3	<i>Aspergillus terreus</i>	2095.71**	648.94**	1395.59**
4	<i>Chrysosporium sp.</i>	1393.846	446.589	826.887
6	<i>Fusarium moniliforme</i>	1294.410	441.355	675.698
7	<i>Penicillium sp.</i>	1062.393	294.815	523.346

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REFERENCES:

- Abd El-Nasser, N. H., Helmy, S. M. and El-Gammal, A. A. (1997). Formation of enzymes by biodegradation of agricultural wastes with white rot fungi. *Polymer Degradation and Stability*, 55: 249-255.
- Adsul, M. G., Ghule, J. E., Shaikh, H., Singh, R., Bastawde, K. B. and Gokhale, D. V. (2005). Enzymatic hydrolysis of delignified bagasse polysaccharides. *Carbohydrate Polymers*, 62: 6-10.
- Bailey, M. J., Biely, P., Poutanen, K. (1992). Interlaboratory testing of methods for assay of xylanase activity. *Journal of Biotechnology*, 23(3): 257-270.
- Barnett, H. L. and Hunter, B.B. (1972). *Illustrated Genera of Imperfect Fungi*. 3rd edition Minneapolis, Minn, USA: Burgess Publishing Company.
- Bataillon, M., Cardinali, A.P.N. and Duchiron, F. (1998). Production of xylanases from a newly isolated alkalophilic thermophilic *Bacillus sp.* *Biotechnology Letters*, 20 (11): 1067-1071.
- Biely, P. (1993). Biochemical aspects of the production of microbial hemicellulases. In: M. P. Coughlan and G. P. Hazlewood (Eds.), *Hemicellulose and Hemicellulases* (pp. 29-51). London: Portland Press.
- Gupta, V.K., Gaur, R., Gautam, N., Kumar, P., Yadav, I.J., and Dharmwal, N.S. (2009). Optimization of xylanase production from *Fusarium solani* F7. *American Journal of Food Technology*, 4(1): 20-29.
- Halttrich, D., Nidetzky, B., Kulbe, K. D., Steiner, W. and Zupancic, S. (1996). Production of fungal xylanases. *Bioresource Technology*, 58: 137-161.
- Kavya, V. and Padmavathi, T. (2009). Optimization of growth conditions for xylanase production by *Aspergillus niger* in solid state fermentation. *Polish Journal of Microbiology*, 58(2): 125-130.
- Knob, A., Beitel, S. M., Fortkamp, D., Terrasan, C.R.F. and De Almeida, A. F. (2013). Production, Purification and Characterization of a Major *Penicillium glabrum* Xylanase using Brewer's Spent grain as Substrate. *BioMed Research International*, 2013, Article ID 728735, 8 pages.
- Kuhad, R.C. and Singh, A. (1993). Lignocellulose biotechnology: current and future prospects. *Critical Reviews in Biotechnology*, 13: 151-172.
- Li, M. A., Soliman, H. M., Sherief, A. D. A. and Tanash, A. B. (2012). Production of xylanase by *Aspergillus niger* and *Trichoderma viride* using some agricultural residues. *International Journal of Agricultural Research*, 7(1): 46-57.
- Mandels, M. and Weber, J. (1969). The production of cellulases. *Advances in Chemistry Series*, 95: 394-414.
- Miller, G.L. (1959). Use of dinitrosalicylic acid reagent for determination of reducing sugar. *Analytical Chemistry*, 31(3): 426-428.
- Murugan, D., Arnold, Pongiya, U.D. and Narayanan, P.M. (2011). Production of xylanase from *Arthrobacter sp.* MTCC 6915 using saw dust as substrate under solid state fermentation. *Enzyme Research*, 2011, Article ID 696942.
- Okafor, U. A., Emezue, T. N., Okochi, V. I., Onyegeme-Okerenta, B. M. and Nwodo-Chinedu, S. (2007). Xylanase production by *Aspergillus niger* ANL 301 using agro-wastes. *African Journal of Biochemistry Research*, 6(14): 1710-1714.
- Pointing, S. B. (1999). Qualitative methods for the determination of lignocellulolytic enzyme production by tropical fungi. *Fungal Diversity*, 2: 17-33.
- Polizeli, M. L. T. M., Rizzatti, C. S., Monti, R., Terenzi, H. F., Jorge, J. and Amorim, D.S. (2005). Xylanase from fungi: properties and industrial applications. *Applied Microbiology and Biotechnology*, 67: 577-91.
- Sonia, K.G., Chadha, B.S. and Saini, H.S. (2005). Sorghum straw for xylanase hyperproduction by *Thermomyces lanuginosus* (D2W3) under solid state fermentation. *Bioresource Technology*, 96: 1561-1569.
- Sohail, M., Siddiqi, R., Ahmad, A. and Khan, S. (2009). Cellulase production from

- Aspergillus niger* MS82: effect of temperature and pH. *New Biotechnology*, 25(6): 437-441.
21. Srinivasan, M.C. and Rele, M.V. (1999). Microbial xylanases for paper industry. *Current Science*, 77: 137-142.
 22. Steiner, W., Lafferty, R. M., Gomes, I. and Esterbauer, H. (1987). Studies on a wild type strain of *Schizophyllum commune*: Cellulase and xylanase production and formation of the extracellular polysaccharide schizophyllan. *Biotechnology and Bioengineering*, 30: 169-178.
 23. Subramanian, S. and Prema, P. (2002). Biotechnology of microbial xylanases: enzymology, molecular biology and application. *Critical Reviews in Biotechnology*, 22 :33-64.
 24. Tachaapaikoon, C., Kyu, K.L. and Ratnakanokchai.(2006). Purification of xylanase from alkaliphilic *Bacillus* sp. K-8 by using corn husk column. *Process Biochemistry*, 41 (12):2441-2445. husk column. *Process Biochemistry*, 41 (12): 2441-2445.