Enzyme Engineering
ISSN: 2329-6674

Bhushan, Enzyme Engg 2012, 1:1
DOI: 10.4172/2329-6674.1000103

Research Article Open Access

Isolation, Screening and Optimized Production of Extracellular Xylanase under Submerged Condition from *Aspergillus Flavus* Mtcc 9390

Bharat Bhushan[1]*, Ajay Pal[2] and Veena Jain[3]

[1]*Central Institute of Post Harvest Engineering and Technology, Abohar, Punjab, India*
[2]*Biochemistry and Nutrition Discipline, Defence Food Research Laboratory, Mysore, India*
[3]*Division of Biochemistry, Chaudhary Charan Singh Haryana Agricultural University, Hisar, India*

Abstract

In order to isolate xylanolytic microbial strains, screening and isolation was done using agricultural waste and decaying biomass. The enzyme super-secreter *Aspergillus flavus* MTCC 9390 was selected for optimized production of xylanase. Various process variables were optimized using conventional 'one-variable-at-a-time' approach which involves varying a single independent variable and maintaining others at a constant level. All culture conditional variables had profound influence on enzyme production and 15-30% increase was brought by nitrogen source only. A synergistic five-fold increase in xylanase production was achieved when an inoculums size of 2 x 10^6 spores/mL was incubated in modified Czapek Dox-A for 6 days at pH 6.0 and temperature 45ºC under static conditions in submerged fermentation.

Keywords: *Aspergillus flavus*; Xylanase; Medium optimization

Introduction

The complex heterogenous polysaccharide after cellulose in cereals, hardwood and fruit is xylan. These hemicelluloses interact with pectin polysaccharides and the aromatic polymer lignin, and integrate with the cellulose fibrils, creating a rigid structure which strengthens the cell wall. They also form covalent cross-links, which are thought to be involved in limiting cell growth and reducing cell wall biodegradability. Structurally, xylan consists of a β-1, 4-linked D-xylose backbone and can be substituted by different side groups such as L-arabinose, D-galactose, acetyl, feruloyl, *p*-coumaroyl, and D-glucuronic acid residues [1].

The biodegradation of the xylan backbone requires two classes of enzymes. Endoxylanases (EC 3.2.1.8) are able to cleave the xylan backbone into smaller oligosaccharides, which can then be degraded further to xylose by β-xylosidase (EC 3.2.1.37). Endoxylanases differ in their specificity towards the xylan polymer. Some enzymes cut randomly between unsubstituted xylose residues, whereas the activity of other endoxylanases strongly depends on the substituents on the xylose residues neighboring the attacked residues.

Production of xylanases from microorganisms has been reported to be both growth associated [2] and non-growth associated [3]. The production potential of xylanase has been found to be influenced by microbe type and its strain [4], nutrient type and concentration, growth conditions [5]. For example eubacteria and archaebacteria produce xylanase having higher temperature optima and better thermostability than those of fungi but the yield of the enzyme produced by these bacteria is comparatively lower than that produced by fungi [6].

Our group is actively engaged in the studies on industrially important microbial proteins [7-12]. The present manuscript, in continuation, describes the optimization of xylanase production from a soil isolate *Aspergillus flavus* MTCC 9390 using submerged fermentation.

Materials and methods

Microorganism and inoculum

Isolation was carried out from soil samples collected from different fruit and vegetable markets of Hisar (Haryana), India. Based on the ability of xylanolysis and potential of xylanase production, a total of nine isolates were screened. Finally one fungal isolate was selected as the potential producer of xylanase which was identified at the Institute of Microbial Technology (IMTECH), Chandigarh, India as *Aspergillus flavus*. It had been included to the collection at IMTECH with accession number MTCC 9390. The inoculum was prepared in potato dextrose medium (PDM) containing xylan as the carbon source, by harvesting spores from 120 h old cultures grown at 30ºC.

Fermentation parameters

Submerged fermentation (SmF) was carried out with 50mL broth of Czapek Dox Modified; CDM-A medium supplemented with organic nitrogen and trace metals having the following composition (gL^{-1}) KH_2PO_4,1.0; $NaNO_3$,2; KCl, 0.5; yeast extract, 0.5; peptone, 0.5; $MgSO_4.7H_2O$, 0.5; $FeSO_4$, 0.01; $ZnSO_4$, 0.001; $CuSO_4$, 0.0005 and xylan, 1gm. in 250mL Erlenmeyer flasks with inoculum size of 1x10^5 spores/mL and incubated at 30ºC for 8 days under static conditions in a BOD incubator (Remi, India). After 8 days, culture broths were centrifuged at 3000 × g for 10min and supernatant was assayed for extracellular xylanase activity.

For maximum production of xylanase, various culture conditions *viz*., carbon and nitrogen source, type of medium, inoculum size, pH, temperature and incubation period were optimized by conventional 'one variable at a time' approach which involves varying a single independent variable at a time while maintaining the others at a constant level. Fungal isolate was grown in different media, containing 0.1% xylan, at pH 6.0 for 192 h. Medium giving maximum growth was

***Corresponding author**: Bharat Bhushan, Central Institute of Post Harvest Engineering and Technology, Abohar, Punjab, India, Tel: +09478-202-080; E-mail: buddingbiochemist@gmail.com

Received February 21, 2012; **Accepted** March 23, 2012; **Published** March 26, 2012

Citation: Bhushan B, Pal A, Jain V (2012) Isolation, Screening and Optimized Production of Extracellular Xylanase under Submerged Condition from *Aspergillus Flavus* Mtcc 9390. Enzyme Engg 1:103. doi: 10.4172/2329-6674.1000103

further used to standardize the inoculum size by inoculating different concentrations (1-5x10^6 spores/mL) of inoculums. Similarly, the temperature and time of incubation were optimized by growing the fungal isolate at different temperatures (25-50°C) for different time periods (72-192 hrs). Different organic *viz*., yeast extract (YE), beef extract (BE), peptone (PPT), tryptone (TPT), casein hydrolysate (CH), cotton leaf residue (CLR), soybean residue (SR) and corn powder (CP) were supplemented separately to a final concentration of 0.3 % (*w/v*) to study the microbial growth and xylanase activity. Glucose, fructose, galactose, arabinose, lactose, sucrose and maltose at a final concentration of 0.1% while wheat straw (WS), oat bran (OB) and birch-wood xylan (BW) were used as the carbon source at a final concentration of 1.0%.

Process variables

Effect of carbon and nitrogen sources and their concentrations, type of medium, inoculum size, pH, incubation temperature and time period, was examined in SmF. Statistical analysis was done to determine the effect of variables on production of xylanase using software 'Statistical Package for Agricultural Scientists' OPSTAT.

Enzyme isolation and assay

After 144 hrs of growth, the fungal broth was filtered through 4 layers of muslin cloth and centrifuged at 3000 × g for 15 min at 4°C in a refrigerated centrifuge. The supernatant taken as enzyme extract containing extracellular xylanase was used to assay the enzyme activity. Briefly, 1 mL of 1% xylan solution (in 0.05M, pH 6.0 sodium citrate buffer) was mixed with 0.1 mL enzyme solution and incubated for 15 min at 60°C. The reaction was stopped by addition of 1 mL of DNS reagent. The mixture is heated for 5 min at 100°C (boiling water bath) and then cooled in cold water. Absorbance of samples was measured at 540 nm against the substrate blank. A standard curve of xylose ranging from 0-1000 µg/mL was prepared and then quantified the released xylose in the samples using standard curve. One unit of xylanase activity is defined as the amount of enzyme liberating 1µmole of xylose equivalent under the experimental conditions in 1 min.

Results and Discussion

In recent years, considerable attention has been paid to the use of micro-organisms in industrial fermentation processes, especially enzyme production. There has been a surge of interest in the production of xylanolytic fungi for their use in food and fruit processing industry. Microbial cultures, regardless of the nature of the end product and the type of bioprocess have certain specific requirements for their growth which have to be optimized for their maximum production. The results on the optimization of culture conditions for the maximum production of xylanase by *A. flavus* MTCC 9390 are presented here.

Identification of xylanase producing microorganism

Out of twenty six fungal isolates, only few isolates showing clear zone (Figure 9) on agar plates containing xylan were selected as xylanase producers. These isolates were further screened based on diameter of clearing zone formation and the maximum production of xylanase on PD (potato dextrose)/ME (malt extract) media with supplement of xylan and used for further studies for optimizing culture conditions to get maximum production of xylanase. The selected fungal isolate in the present investigations had been identified at Institute of Microbial Technology (IMTECH), Chandigarh as *Aspergillus flavus* and was included to their collection at the centre with accession number MTCC 9390.

Optimization of culture conditions

Medium: To obtain maximum xylanase production, fungal isolate was grown on different media apart from PDA/ME media *viz.* Xylanase cultivation medium having xylan as a sole carbon/energy source (XCM), Czapek-Dox medium (CDM) and its modified versions (CDM- A, B, C) with 0.1% xylan under SmF. Each medium was inoculated with 1 mL inoculum containing 1x10^6 spores at 30°C for 8 days under static condition in BOD incubator and enzyme production was determined. Maximum activity was observed in modified Czapek Dox medium-A (CDM-A), which was used for further experiments (Figure 1). Xylanase cultivation medium which contained Mg^{2+} ions only showed 73% activity as compared to that in Czapek-Dox medium which contained both Fe^{2+} and Mg^{2+} ions. Further, there are reports [13-15] which suggest that organic source of nitrogen support the maximum enzyme production. Keeping this in view, the Czapek-Dox medium was modified with respect to trace metal ions and nitrogen source. Sodium nitrate was replaced with yeast extract and peptone (replaced the inorganic nitrogen source with the organic one) and Zn^{2+} and Cu^{2+} ions were added in various proportions in the modified Czepek-Dox media to supplement the medium with trace metals. The results shown in Figure 2 depicted that modified Czapek-Dox media-A (CDM-A) which contained metal ions in highest concentration (0.1%) and yeast extract and peptone (0.05% each) along with sodium nitrate (0.2%) showed the maximum xylanase production (17.09 UmL^{-1}). The possible explanation for improvement in growth and enzyme activity at lower concentration of organic nitrogen could be that higher amount of organic nitrogen sources produce more protease activity which may degrade xylanase [16].

Yeast extract and peptone has been used and recommended by many workers [2,12] as nitrogen source. Among inorganic and complex nitrogen sources, yeast extract supported the maximum enzyme production [14]. Though all related inorganic nitrogen sources supported the enzyme production but not as efficiently as yeast extract. Inorganic nitrogen such as $NaNO_3$ (0.5%) has been reported to be the best in stimulating xylanase production by *Cohliobolus sativus* [13].

Inoculum concentration/size: Each 250 mL Erlenmeyer flask containing 50 mL modified CDM-A (pH 6.0) was inoculated with different sizes of working inoculum ranging from 1-5 mL taken

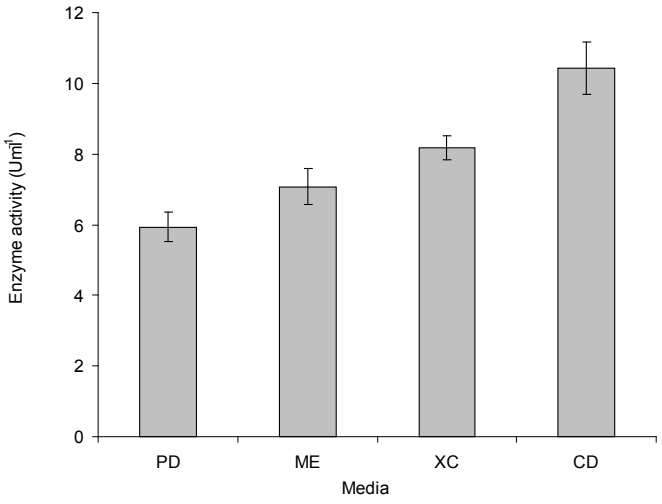

Figure 1: Production of xylanase production by *A. flavus* MTCC9390 on different media.

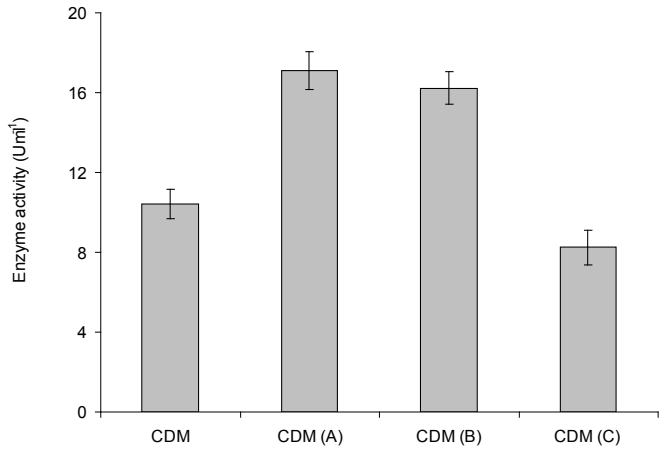

Figure 2: Effect of modified Czapek-Dox media on xylanase production by *A. flavus* MTCC9390.

from spore stock prepared in Tween-80 (approx.1-5x10^6 spores/mL) in a BOD incubator at 30°C for 8 days to determine the optimum inoculum size for xylanase production. Since 2 mL inoculum gave the maximum activity of xylanase, this size was used for further optimization process (Figure 3). Xylanase production from *A. niger* USM A1I and *A. japonicus* was reported to be maximum using 1x10^5 spores/mL and 1x10^7 spores/mL [17,18]. During the cultivation of *A. foetidus* maximum xylanase activity occurred when inoculum had a concentration of 1.5x10^8 spores/mL [19].

pH: To obtain maximum xylanase production by *A. flavus* MTCC 9390, each Erlenmeyer flask containing 50 mL modified CDM-A with pH ranging from 4.0-7.0 was incubated at 30°C with 2 mL inoculum for 8 days. After 8 days of incubation, xylanase activity was determined. Growth medium having pH 6.0 gave the maximum activity and was used for further studies (Figure 4). On either side of the optimum pH (pH 6.0) of the medium, the enzyme production decreased. The initial pH has been observed to influence the transport of enzymes across the cell membrane [13]. The growth and enzyme production by this organism has been reported to be adversely affected at alkaline pH. Similar results has been reported in *A. terreus* where lowest (pH 3.0 or 10.0), moderate (pH 4.0) and maximum xylanase activities (pH 6.0) were observed [20].

The effect of initial culture pH on xylanase production in *P. thermophila* has been reported and found maximum activity when the initial pH was adjusted to 7.0 [21]. High level of xylanase production by this strain was observed in the range of pH 5.0-8.0. *Aspergillus* sp. RSP-6 was active in xylanase production over a broad range of pH from 2.0-6.0 with maximum production at pH 3.0 [14]. The enzyme production was drastically reduced at neutral pH and no enzyme production was noticed in alkaline medium of pH 8.0. A pH of around 5.0, in general, has been observed to be optimum for xylanase production [1,19,22,23].

Incubation period: To study the effect of incubation period, each 250 mL flask containing 50 mL modified CDM-A (pH 6.0) was incubated at 30°C with 2x10^6 spore inoculum. After 72 hrs of incubation, the samples were harvested at regular interval of 24 hrs up to 8 days to determine xylanase activity in the supernatant of each sample. Maximum xylanase production was observed when incubated for 6 days (Figure 5). After 6 days, a decline in the activity was observed reaching a value of 16.38 UmL^{-1} on 8th day of incubation could be due to the release of xylanase from bound substrate or from the autolysis of fungal cells. Similar results were reported in *P. oxalicum* which produced maximum xylanase production after 6 days [15]. Maximum xylanolytic activity in *A. terreus* was observed after 4 days at 35°C and remained at that level until 6.5 days when the activity started to decrease [14]. In *F. solani,* enzyme production started after 24h of inoculation but showed maximum production on 6th day of incubation period at 30°C [22]. Maximum xylanase production by *Aspergillus* sp. RSP-6 occurred on 5th day of incubation and further increase in fermentation time resulted in reduction of activity [14].

Incubation temperature: To determine the optimum temperature for maximum xylanase production by *A. flavus* MTCC 9390, each Erlenmeyer flask containing 50 mL modified CDM-A (pH 6.0) was inoculated with 2x10^6 spores and incubated for 8 days at different temperatures *viz.*, 25, 30, 35, 40, 45 and 50°C in BOD incubator. Xylanase activity determined for 8 days of incubation was found to be maximum at 45°C (Figure 6). Further optimization was done by growing the

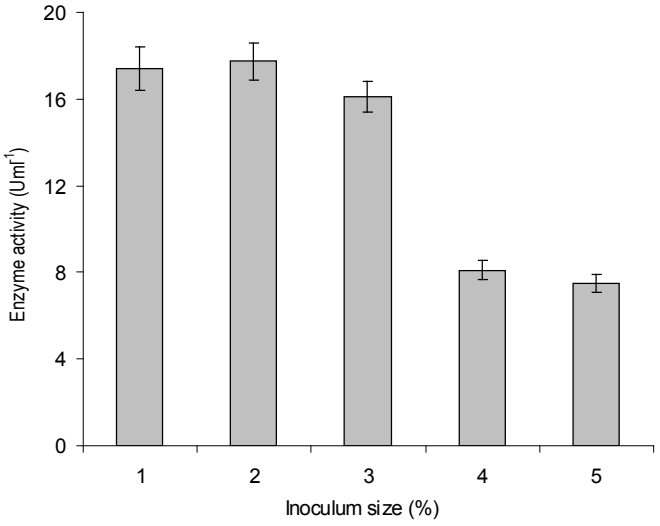

Figure 3: Effect of inoculum size (spore concentation) on xylanase production by *A. flavus* MTCC9390.

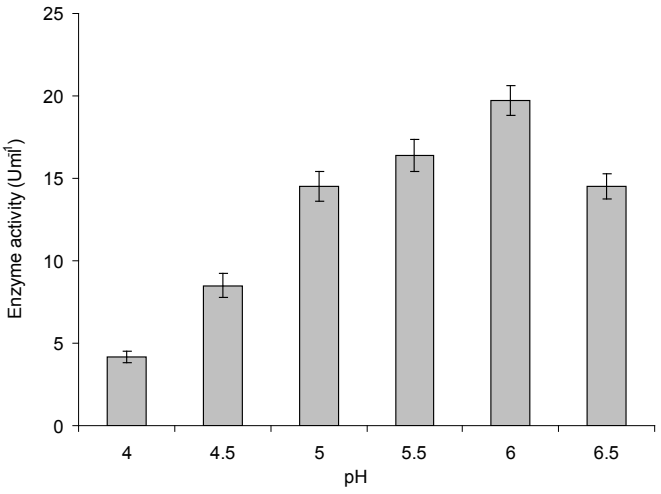

Figure 4: Effect of broth pH on xylanase production by *A. flavus* MTCC9390.

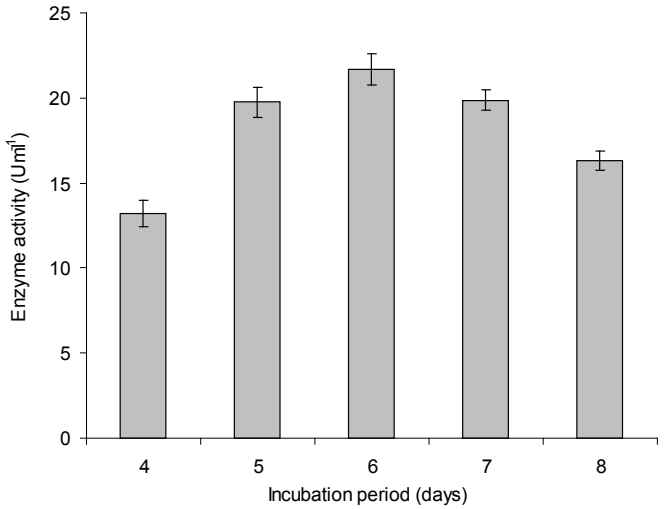

Figure 5: Effect of culture incubation time on xylanase production by *A. flavus* MTCC9390.

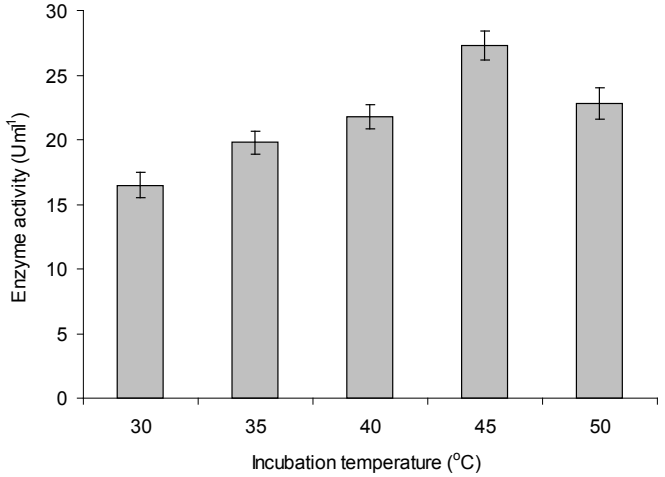

Figure 6: Effect of culture incubation temperature on xylanase production by *A. flavus* MTCC9390.

culture at 45°C. Although the physiological changes induced by high temperatures during enzyme production is not very clear, it has been suggested that at high temperatures, microorganisms may synthesize reduced number of proteins that are probably essential for growth and other physiological processes including enzyme production [24]. By applying the temperature shift during laboratory cultivation, hydrolytic activity could almost be doubled, whereas the xylanolytic production was three fold higher in comparison to cultivation at a constant temperature of 28°C [25]. The ambient temperature of 28 ± 3°C was found to be suitable for maximum production of xylanase in *A. niger* [17].

The best temperatures for xylanase production by *A. japonicum* have been reported to be 25°C [18]. With cultivation temperature lower and higher than the optimum, decline in xylanase activity has been reported [17,22]. A slightly higher temperature of 45°C and 50°C has been reported to be optimum for xylanase production by *P. oxalicum* and *T. aurantiacus*, respectively [15,26].

Carbon source: Different carbon sources were used to determine their effect on xylanase production. Each 250 mL Erlenmeyer flask containing 50 mL modified CDM-A (pH 6.0) contained either of these carbon sources *viz.* various commercially available sugars, wheat straw, oat bran, birch-wood xylan at a concentration of 0.1-1% each. The medium was inoculated with 2 mL inoculum and incubated at 45°C for 6 days and xylanase production was determined. Birch-wood xylan and oat bran at a concentration of 1% showed almost similar xylanase production which was 27.28 and 25.54 UmL^{-1}, respectively (Figure 7). Though, commercial xylan also supported the xylanase production but not as efficiently as wheat straw. None of the monosaccharides and disaccharides tested *viz.* glucose, fructose, galactose, arabinose, maltose, lactose and sucrose was found suitable for xylanase production. Since no xylanase activity could be observed in the absence of xylan in the culture medium, it could be suggested that growing of *A.flavus* in xylan enriched media exerted some effect on enzyme secretion.

Induction of the synthesis of xylan-degrading enzymes by xylanolytic organisms cultured with xylan as carbon source is well documented [4]. However, xylanase production in *Aspergillus* sp. RSP-6 to be constitutive in nature and none of the monosaccharide or disaccharide improved the xylanase production compared to palm fiber as carbon source [14]. In contrast, xylanase activity in *A. pullulans* Y-2311-1 was induced by xylose and xylan [27]. Induction of xylanase activity in *T. fusca* and *P. bryantii* by glucose, cellobiose, water soluble xylan and acid-ethanol soluble xylan has been reported [28,29]. Suppression of xylanase synthesis by readily metabolizable sugars such as glucose and/or xylose has been reported in *Streptomyces* sp. [30].

The use of wheat straw and wheat bran as a carbon source for xylanase production has been reported [13,22,31]. Other agro-residues such as rice straw [26], sugarcane bagasse [32], corncob [12,33,34], oatspelt xylan [15] and Brewer's spent grain [35] have also been reported as suitable substrates for xylanase production.

Nitrogen source: Effect of different nitrogen sources *viz.*, tryptone, casein hydrolysate, soybean residue, cotton leaf residue and corn powder on xylanase production was studied by replacing yeast extract by various nitrogen sources in modified CDM-A (pH 6.0). Each 250 mL Erlenmeyer flask containing 50 mL medium (pH 6.0) was incubated with 2 mL inoculum, containing $2x10^6$ spores, at 45°C for 6 days under static conditions, after which xylanase production was

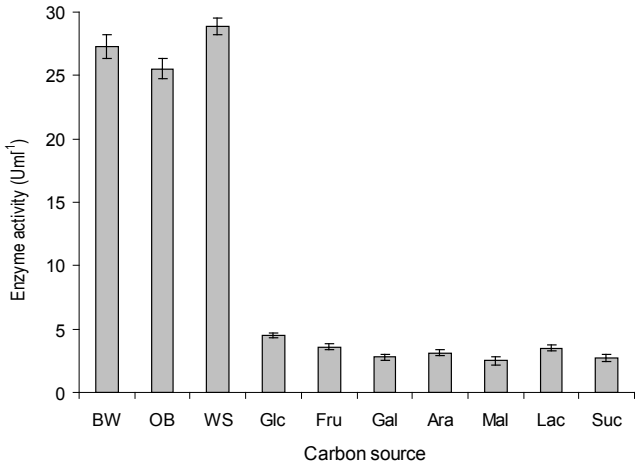

Figure 7: Effect of carbon source on xylanase production by *A. flavus* MTCC9390.

determined. Corn powder was found to be the best nitrogen source as it gave the maximum xylanase activity (Figure 8). Further to find out its optimum concentration, different concentrations of corn powder were ranging from 0.1–0.5% in modified CDM-A (pH 6.0) containing xylan as carbon source was investigated. At 0.1% concentration, xylanase production was found to be maximum (data not shown). The results obtained during the present investigations are in agreement with those reported [14] where yeast extract has been reported to be the best nitrogen source for xylanase production by *Aspergillus* sp. RSP-6 and on contrary, nitrogen sources such as peptone, beef extract, soybean meal, peanut meal and corn steep liquor were found to be poor nitrogen sources.

Among the other organic nitrogen sources, defatted rapeseed meal induced maximum enzyme production by *Streptomyces* sp. [36]. Corn steep liquor and soya bean meal produced maximum xylanase level in *B. licheniformis* A 99 [3]. In contrast to our results, peptone as the best source of organic nitrogen for the production of xylanase from *A. niger, F. solani* and *T. harzianum* [13,22,37]. However, when alternative nitrogen sources such as cotton leaf residues and soybean residues were used individually and in combination, lower xylanase activities were observed [37].

The medium optimized at this stage for xylanase production by *A. flavus* MTCC 9390 was termed as 'xylanase overproduction medium'

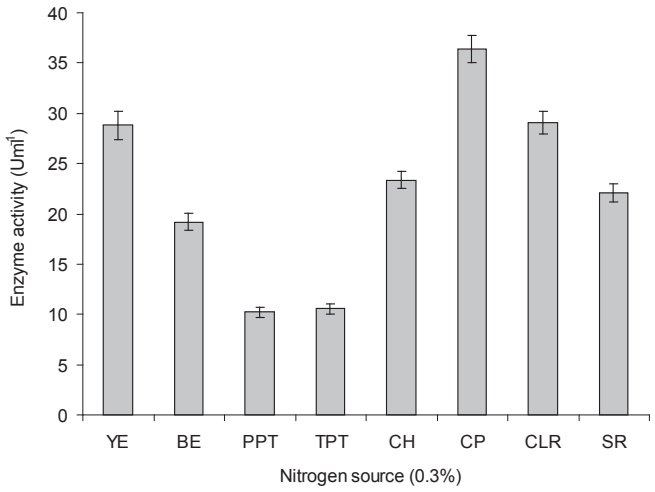

Figure 8: Effect of nitrogen source on xylanase production by *A. flavus* MTCC9390.

Figure 9: Agar plate showing clear zones of hydrolysis formed by xylanolytic isolates.

and finally, optimized conditions were: inoculum size of 2 mL with 6 days of incubation in CDM-A medium of pH 6.0 at 45°C.

Conclusion

Isolation and multi-step screening method employed in the study led to the isolation of one efficient strain culture of *A. flavus* for production of xylanase. Results obtained on optimization of process variables under SmF revealed that wheat straw and corn powder could be effectively used for xylanase production. About five fold enhancement in xylanase production by *A. flavus* MTCC 9390 was achieved when an inoculum size of $3x10^6$ spores/mL was incubated in modified Czapek-Dox medium-A at pH 6.0 and 45°C for 144 hrs. The low cost of its production and acidophilic nature of enzyme may further broaden the scopes for its use in fruit juice industries.

Acknowledgement

The authors are grateful to the CSIR-UGC for financial assistance and to the Head, Department of Biochemistry, Chaudhary Charan Singh Haryana Agricultural University for providing all basic facilities and guidance during this investigation.

References

1. Subramaniyan S, Prema P (2002) Biotechnology of microbial xylanases: enzymology, molecular biology, and application. Crit Rev Biotechnol 22: 33-64.
2. Sa-Pereira P, Mesquita A, Duarte JC, Barros MRA, Costa-Ferreira M (2002) Rapid production of thermostable cellulase free xylanase by a strain of *Bacillus subtilis* and its properties. Enzyme Microb Technol 30: 924-933.
3. Archana A, Satyanarayana T (1997) Xylanase production by thermophilic *Bacillus licheniformis* A99 in solid-state fermentation. Enzyme Microb Technol 21: 12-17.
4. Collins T, Gerday C, Feller G (2005) Xylanases, xylanase families and extremophilic xylanases. FEMS Microbiol Rev 29: 3-23.
5. Azin M, Moravej R, Zareh D (2007) Production of xylanase by *Trichoderma longibrachiatum* on a mixture of wheat bran and wheat straw, optimization of culture condition by Taguchi method. Enzyme Microb Technol 40: 801-805.
6. Haltrich D, Preiss M, Streiner W (1993) Optimization of culture medium for increased xylanase production by a wild strain of *Schizophyllum commune*. Enzyme Microb Technol 15: 854-860.
7. Pal A, Khanum F (2010) Production and extraction optimization of xylanase from *Aspergillus niger* DFR-5 through solid-state-fermentation. Bioresour Technol 101: 7563-7569.
8. Pal A, Khanum F (2011) Covalent immobilization of xylanase on glutaraldehyde activated alginate beads using response surface methodology: Characterization of immobilized enzyme. Process Biochem 46: 1315-1322.
9. Pal A, Khanum F (2011) Identification and optimization of critical medium components using statistical experimental designs for enhanced production of xylanase from *Aspergillus flavus* DFR-6. Food Technol Biotechnol 49: 228-236.
10. Pal A, Khanum F (2011) Purification of xylanase from *Aspergillus niger* DFR-5: individual and interactive effect of temperature and pH on its stability. Process Biochem 46: 879-887.
11. Pal A, Ramana K V (2009) Isolation and preliminary characterization of a nonbacteriocin antimicrobial compound from *Weissella paramesenteroides* DFR-8 isolated from cucumber *(Cucumis sativus)*. Process Biochem 44: 499-503.
12. Pal A, Ramana K V (2010) Purification and characterization of bacteriocin from *Weissella paramesenteroides* DFR-8, an isolate from cucumber *(Cucumis sativus)*. J Food Biochem 34: 932-948.
13. Bakri Y, Jawhar M, Arabi MIE (2008) Improvement of xylanase production by *Cochliobolus sativus* in submerged culture. Food Technol Biotechnol 46: 116-118.
14. Sathish T, Lakshmi GS, Rao ChS, Brahmaiah P, Prakasham RS (2008) Mixture design as first step for improved glutaminase production in solid-state fermentation by isolated *Bacillus*. Lett Appl Microbiol 47: 256-262.
15. Muthezhilan R, Ashok R, Jayalakshmi S (2007) Production and optimization

of thermostable alkaline xylanase by *Penicillium oxalicum* in solid state fermentation. Afr J Microbiol Res pp.20-28.

16. Anthony T, Raj KC, Rajendran A, Gunasekaran P (2003) Inhibition of proteases during fermentation improves xylanase production by alkali tolerant *Aspergillus fumigatus* ARI. J Biosci Bioeng 96: 394-396.
17. Kheng PP, Omar IC (2005) Xylanase production by a local fungal isolate, *Aspergillus niger* USM AI 1 via solid state fermentation using palm kernel cake (PKC) as substrate. Songklanakarin J Sci Technol 27: 325-336.
18. Simoes MLG, Tornisielo SMT (2005) Optimization of xylanase biosynthesis by *Aspergillus japonicus* isolated from a "Caatinga" area in the Brazilian State of Bahia. Afr J Biotechnol 5: 1135-1141.
19. Shah AR, Madamwar D (2005) Xylanase production by newly isolated *Aspergillus foetidus* strain and its characterization. Process Biochem 40: 1763-1771.
20. Chidi SB, Godana B, Ncube I, vanRensburg E J, Cronshaw A, et al. (2008) Production, purification and characterization of cellulase-free xylanase from *Aspergillus terreus* UL 4209. Afr J Biotechnol 7: 3939-3948.
21. Yang SQ, Yan QJ, Jiang ZQ, Li LT, Tian HM, et al. (2006) High-level of xylanase production by the thermophilic Paecilomyces themophila J18 on wheat straw in solid-state fermentation. Bioresour Technol 97: 1794-1800.
22. Gupta VK, Gaur R, Gautam N, Kumar P, Yadav IJ, et al. (2009) Optimization of xylanase production from *Fusarium solani* F7. American Journal of Food Technology 4: 20-29.
23. Sridevi B, Charya MAS (2011) Isolation, identification and screening of potential cellulase-free xylanase producing fungi. African Journal of Biotechnology 10: 4624-4630.
24. Gawande PV, Kamat MY (1999) Production of *Aspergillus xylanase* by lignocellulosic waste fermentation and its application. J Appl Microbiol 87: 511-519.
25. Smit JP, Rinzema A, Tramper J, vanSonsbeek HM, Hage JC, et al. (1998) The influence of temperature on kinetics in solid-state fermentation. Enzyme Microb Technol 22: 50-57.
26. Dhillon A, Gupta JK, Jauhari BM, Khanna SA (2000) A cellulase poor, thermostable, alkali-tolerant xylanase produced by *Bacillus circulans* AB16 grown on rice straw and its application in biobleaching of eucalyptus pulp. Bioresour Technol 73: 273-277.
27. Li XL, Ljungdahl LG (1994) Cloning, sequencing, and regulation of a xylanase gene from the fungus Aureobasidium pullulans Y-2311-1. Appl Environ Microbiol 60: 3160-3166.
28. Chen S, Wilson DB (2007) Proteomic and transcriptomic analysis of extracellular proteins and mRNA levels in Thermobifida fusca grown on cellobiose and glucose. J Bacteriol 189: 6260-6265.
29. Miyazaki K, Hirase T, Kojima Y, Flint HJ (2005) Medium- to large-sized xylo-oligosaccharides are responsible for xylanase induction in Prevotella bryantii B14. Microbiology 151: 4121-4125.
30. Beg QK, Bhushan B, Kapoor M, Hoondal GS (2000) Enhanced production of a thermostable xylanase from Streptomyces sp. QG-11-3 and its application in biobleaching of eucalyptus kraft pulp. Enzyme Microb Technol 27: 459-466.
31. Okafor UA, Okochi VI, Onyegeme-okerenta BM, Nwodo Chinedu S (2007) Xylanase production by *Aspergillus niger* ANL 301 using agro-wastes. African Journal of Biotechnology 6: 1710-1714.
32. Sandrim VC, Rizzatti ACS, Terenzi HF, Jorge JA, Milagres AMF, et al. (2005) Purification and biochemical characterization of two xylanases produced by *Aspergillus caespitosus* and their potential for kraft pulp bleaching. Process Biochem 40: 1823-1828.
33. da Silva R , Lago ES, Merheb CW, Macchione MM, Park YK, et al. (2005) Production of xylanase and CMcase on solid state fermentation in different residues by *Thermoascus aurantiacus* miehe. Braz J Microbiol 36: 235-241.
34. Gomes J, Purkarthofer H, Hayn M, Kapplmuller J, Sinner M, et al. (1993) Production of a high level of cellulase-free xylanase by the thermophilic fungus *Thermomyces lanuginosus* in laboratory and pilot scales using lignocellulosic materials. Appl Microbiol Biotechnol 39: 700-707.
35. Terrasan CR, Temer B, Duarte MC, Carmona EC (2010) Production of xylanolytic enzymes by *Penicillium janczewskii*. Bioresour Technol 101: 4139-4143.
36. Elegir G, Szakacs G, Jeffries TW (1994) Purification, Characterization, and Substrate Specificities of Multiple Xylanases from Streptomyces sp. Strain B-12-2. Appl Environ Microbiol 60: 2609-2615.
37. Seyis I, Aksoz N (2003) Determination of some physiological factors affecting xylanase production from Trichoderma harzianum 1073 D3. New Microbiol 26: 75-81.
38. Schneider G, Strehaiano P, Taillandier P (2001) Improvement of fed-batch process for high level xylanase production by a *Bacillus* strain. J Chem Technol Biotechnol 76: 456-460.