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ENHANCEMENT OF XYLANASE ACTIVITY ON DIFFERENT CULTURAL CONDITIONS OF *ASPERGILLUS NIGER* IN SUBMERGED CULTURE

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Abstract

Enhancement of xylanase activity in *Aspergillus niger* was obtained by changing the growth parameters during present investigation. Previously isolated strain of *A. niger* was cultivated by optimizing the culture conditions in submerged culture for enhanced xylanase activity using low cost lignocellulosic waste like wheat bran, sugarcane baggase, wheat straw, corn cobs and rice straw. Highest activity of xylanase (25.49 U/ml) was observed when fungus was grown on media containing wheat bran as carbon source. The influence of temperature, pH, salt concentrations and incubation period on activity of xylanase was also observed. The maximum activity (36.06 ± 0.148 U/ml) was recorded on day 7th at 3 percent salt concentration and optimum temperature of 45ºC and 5.5 pH. It has been found that *A. niger* could be the important fungus for conversion of lignocellulosic waste into useful products.

Key words: *Aspergillus niger*, xylanase, submerged culture, lignocellulosic waste, wheat bran.

Introduction

Plants cell wall is consists of cellulose, hemicellulose and pectin materials of which hemicellulose is more complex heterogenous substance consist of its basic structural unit xylan, which is a branched heteropolysaccharide composed of a backbone of β-1-4 xylopyranosyl units and different side groups like D-galactose, L-arabinose, feruloyl and glucouranyl acetyl residues (Shallom and Soham, 2003). Xylanases are the class of enzyme able to hydrolyze the xylan into oligosaccharide and D- xylose. Because of complex structure of xylan several side chains cleaving enzymes required for its complete hydrolysis of which endo 1-4 β-xylanase and β- xylosidase are key enzymes responsible for breakdown of the xylan backbone (Thomas *et al*., 2014). Endoxylanase attack on xylan to produce xylooligosaccharides and the β-xylosidase hydrolyze xylobiose and xylotriose to xylose and other monomers.

Xylanase are found to produced by various microorganisms like fungi, bacteria, actinomycetes and yeast. Among the different sources of xylanase, filamentous fungi are widely used for commercial production of the enzyme because they are more capable to produce higher level of extracellular xylanase into the medium (Sakthiselvan *et al.,* 2014) as compared to yeast and bacteria.

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In the last few decades the biotechnological applications of xylanase seeking greater attention of researchers. The use of xylanase in the bioconversion of lignocellulosic waste is the focus of several studies because of the xylose production which is used as a raw material for the cultivation of single cell protein and in the production of xylitol, xylonic acid and ethanol. A major application of xylanase is in paper and pulp industry as a biobleaching agent where xylanse could reduce the demand of chlorine which causes environmental pollution (Singh *et al.,* 2013). A part from this xylanase also have applications in animal feed industry, food industry, textile and pharmaceutical industry (Pedersen *et al*., 2015). Submerged fermentation (SmF) technique has been used traditionally for the cultivation of useful enzymes because of easy handling and maintenance of homogenous conditions throughout the experiment (Guleria *et al.,* 2013). However, the productivity of enzyme in solid state fermentation (SSF) technique is much higher and can also minimize the production cost (Hui *et al*., 2010).

Commonly filamentous fungi are used to produce

xylanase and improvement in enzyme yields and activity has been achieved by modifying cultural parameters or by mutation (Singh *et al.,* 1995) and hybridization. During present study we reports on enhancement of activity of xylanase in *Aspergillus niger* by modifying its cultural conditions.

Material and Methods

Chemicals used

The chemicals used in this experiment were of analytical grade and purchased from Himedia, Merck and SRL. Lignocellulosic waste (wheat straw, sugarcane baggase, wheat bran, corn cobs and rice straw) were arranged from local market.

Selection of experimental organisms

The fungal isolates *Aspergillus niger* used in this study was isolated previously from the soil of agricultural field. Being one of the best xylanse producing fungi it was selected for further studies. The culture of *Aspergillus niger* was maintained and preserved on YPSs medium on agar slants at 4ºC.

Cultivation of enzyme through submerged culture

The production of enzyme through submerged culture was undertaken in an Erlenmeyer flasks of 250 ml containing 60 ml of Bhatt and Maheshwari medium (1987). The medium composition in g/l was $CoCl_2.6H_2O$, 0.02; $NH_4H_2PO_4$, 2.0; KH_2PO_4, 3.0; $MgSO_4$ $7H_2O$, 0.5; K_2HPO_4, 2.0; $CaCl_2$. $2H_2O$, 0.1; $MnSO_4$, $4H_2O$, 0.016, $FeSO_4$, $7H_2O$, 0.05; $ZnSO_4.7H_2O$, 0.0143, Peptone, 1.0; Yeast extract 1.0, in which carbon source was supplemented with 1% sugarcane baggase. After sterilizing the medium flasks were inoculated by 1ml spore suspension of test fungus. The incubation was carried out on a rotary shaker at 120 rpm and 28 ± 2ºC for 120 hours. After termination of the required period centrifuged the broth at 6000 rpm for 15 min in order to remove unwanted residues and the supernatant thus obtained was treated as enzyme.

Xylanase assay

The beech wood xylan was used as a substrate for the assay of enzyme activity. The reaction mixture was prepared by adding 1.8 ml substrate (1% xylan) and 0.2 ml of enzyme and keeping it in a water bath for 30 min at 50ºC (Bailey *et al*., 1992). 1 ml of above reaction mixture was taken after incubation and 3 ml of DNS reagent added to it, shaken well boiled for 5 minutes. Reading was taken after cooling the mixture at 540 nm against spectro zero. Enzyme activity was calculated by determining the reducing sugar against the standard of xylose (Miller and Khan, 1959). One unit of enzyme activity was defined as the amount of enzyme required for hydrolyzing 1µmol of xylose per minute under the assay conditions.

Optimization of cultural parameters

The optimization of cultural conditions was undertaken by modification of different parameters in a stepwise manner for production of xylanase. The effect of incubation period was undertaken by taking the enzyme activity of *A.niger* at every 24 hour interval for 7 days.

The test organism was grown on different lignocellulosic waste i.e. sugarcane baggase, wheat bran, rice straw, corn cobs and rice bran. 1 gm substrate of each was added in 100 ml Erlenmayer flask separately containing 25 ml of medium and incubated at 28±2 for 7 days.

Temperature effect was examined by growing test organisms on different temperature ranging from 35ºC to 60 with an interval of 5ºC each for growth while effect of temperature on enzyme assay was found out by detecting enzyme activity at different incubation temperature from 35 to 80ºC with the help of DNS method as mentioned above.

The effect of pH on enzyme activity was observed by changing pH of the medium from 4.0-9.0 in citrate phosphate buffer, keeping other conditions as optimum. The optimization of different salt concentration was examined by growing test organism on standard medium with different salt concentration *i.e.* from 1.0 to 5.0 percent. Xylanase activity in optimized conditions was compare with that of its activity during unoptimized conditions.

The activity of xylanase was calculated by taking standard graph of xylose at 540 nm. Results obtained from the above experiments were shown as their mean ± standard deviation of triplicates.

Result and Discussion

Submerged cultivation of xylanase using lignocellulosic waste was proved to be a potential technique because of sufficient oxygen supply, availability of more nutrients and least time requirement for growth than other techniques. The synergistic effect of several xylan decomposing enzymes could also be observed

Table 1: Xylanase activity of *Aspergillus niger* at different incubation periods on wheat bran.

Incubation period (Days)	Enzyme activity (U/ml)
3rd	10.96±0.681
5th	16.49±0.406
7th	22.19±0.286
9th	18.89±0.649

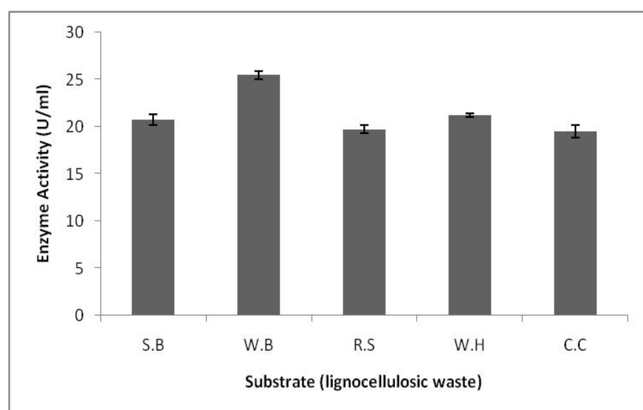

Fig. 1: Xylanase activity of *A. niger* on different substrate. S.B= sugarcane baggase, W.B= wheat bran, R.S= rice straw, W.H= wheat husk, C.C= corn cobs.

during submerged culture which results in better utilization of biomass for improved xylanase production (Bajpai, 2014; Polizeli *et al.,* 2005).

The activity of xylanase on different incubation period was examined and result showed that the maximum activity (22.19 U/ml) was achieved at 7th day of incubation (Table 1) while at 3rd day of incubation the minimum activity was observed. A notable decline of enzyme production was observed after 7th day of incubation. This might be due to the nutrient depletion and aggregation of toxic wastes in culture media. The result obtained indicates that among the different substrate used wheat bran proved to be the best substrate for xylanase production followed by sugarcane baggase (Fig. 1). Rice straw had been proved as poor substrates for the xylanase production by *A. niger.*

Wheat bran is a cheapest substrate for fermentation and contain highest amount of xylan and proved to be a promising lignocellulosic substrate for xylanase cultivation in submerged cultivation. It is also believed that wheat bran particles have decomposed and form soluble compound could be utilized by the fungus. The highest activity of xylanase in media having wheat bran as a single carbon source was also obtained by many workers (Okafor *et al.*, 2007; Padmavathi *et al.*, 2009; Muthezhilan *et al.*, 2007; Batailon *et al.*, 2000), however lowest xylanase production on wheat bran was observed by *Bacillus lichenformis* (Archana and Satyanarayana, 1997; Zychlinski *et al.*, 1994).

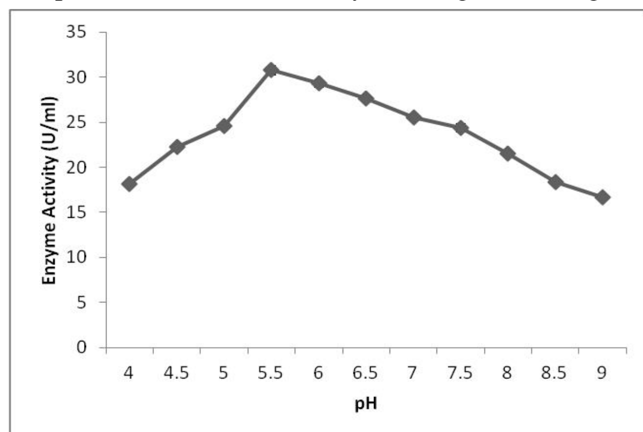

Fig. 2: Effect of pH on xylanase activity of *A. niger* at optimum temperature on wheat bran.

Table 2: Xylanase activity of *Aspergillus niger* at different temperature at optimum incubation period on wheat bran.

Temperature (ºC)	Enzyme activity (U/ml)
30	16.29 ± 0.532
35	17.4 ± 0.466
40	18.49 ± 0.399
45	27.40 ± 0.412
50	24.40 ± 0.443
55	23.37 ± 0.294
60	20.46 ± 0.386

Temperature range from 30 to 60ºC was selected for optimization of temperature for production of xylanase. Results obtained indicate that optimum cultivation temperature was 45ºC at which maximum enzyme activity (27.40 U/ml) was achieved by the fungus (Table 2). While highest activity of xylanase was noticed at 50ºC of incubation temperature. A notable decrease in activity was also seen thereafter. Growth rate as well as enzyme production both are affected dramatically by cultivation temperature. The *A. niger* is a mesophilic fungus however the optimum temperature for maximum activity was at thermophilic range. The temperature generated through metabolic activities of the organisms also influence their cultivation temperature because the growth of fungus at the temperature below and above the optimum was inhibited thus the enzyme activity could also be decreased (Yaun and Rangyu, 1999; Rahman *et al.,* 2003).

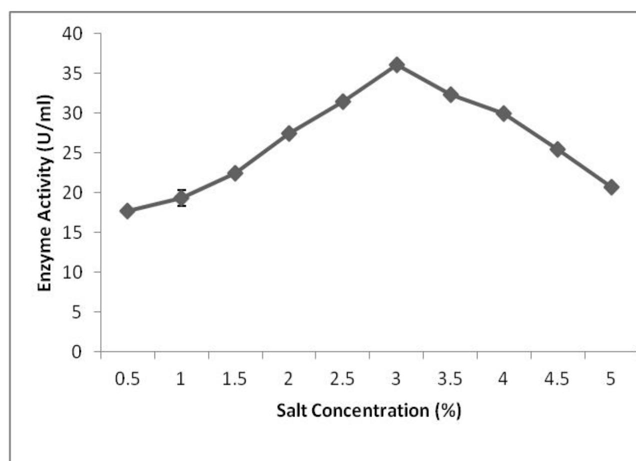

Fig. 3: Effect of different salt concentration on xylanase activity of *A. niger* at optimum temperature and pH on wheat bran.

Optimum pH was explored by growing test organism on different pH from 4.0 to 8.0 which was found as pH 5.5 (30.75 U/ml). The pH optima for xylanase activity *i.e.* for enzyme substrate reaction were found to be 6.5. The pH of a medium effectively influences enzymatic activities and movement of various substances across the cell membrane (Kuhad, Kapoor and Nair, 2008). There are several reports on production and characterization of thermostable and alkali tolerant xylanase (Bim and Franco, 2000; Tseng *et al*., 2002; Dhillon *et al*., 2000).

Effect of salt concentration was also determined on xylanase activity which was highest (36.06 U/ml) at 3% of salt concentration (Fig. 3). Similar result was also obtained by Muthezhilan *et al.,* (2007). Activity was found to be increase with increase in salt concentration but upto a limit because at higher salt concentration the ions of salt can denature the enzyme. Double salt optima were observed for *Thermoascus aurantiacus* at 1.5% and 2.5 % (Yadav and Jaitly, 2011). The study of xylanase profile before and after optimization of growth conditions showed that higher production of xylanase was recorded at optimized conditions as compare to unoptimized conditions. It was observed from this study that xylanase production could also be enhanced by modifying their ecological parameters in order to make it more suitable for industrial purpose. *Aspergillus niger* proved to be a potential fungal strain for production of xylanase on optimized submerged fermentation conditions using cheaper lignocellulosic waste.

References

Archana, A. and T. Satyanarayana (1997). Xylanase production by thermophilic *Bacillus lichenformis* A99 in solid state fermentation. *Enzyme Microbiol. Technol*., **21:** 12-17.

Bailey, M.J., P. Biely and K. Poutanen (1992). Interlaboratory testing of methods for assay of xylanase activity. *J. Biotechnol*., **23(3):** 257- 270.

Bajpai, P. (2014). Sources, production and classification of xylanases. Xylanolytic Enzyme Academic Press (Imprint Elsevier), Tokyo, 43-52.

Bataillon, M., A.P. Nunes Cardinali, N. Castillon and F. Duchiron (2000). Purification and characterization of a moderately thermostable xylanase from *Bacillus sp.* strain SPS-0. *Enzyme Microbiol. Technol*., 187-192.

Bhat, K.M. and R. Maheshwari (1987). *Sporotrichum thermophile* growth cellulose degradation and cellulase activity. *Appl. Environ. Microb.,* **53:** 2175-2182.

Bim, A. and T. Franco (2000). Extraction in aqueous two phase systems of alkaline xylanase produced by *Bacillus plumilus* and its application in kraft pulp bleaching. *J. chrom*., **743:** 349-356.

Dhillon, A., J.K. Gupta, B.M. Jauhari and S. Khanna (2000). A cellulase-poor, thermostable, alkalitolerent xylanase produced by *Bacillus circulans* AB 16 grown on rice straw and its application in biobleaching of eucalyptus pulp. *Bioresour. Technol*., **73:** 273-277.

Guleria, S., A. Walia, A. Chauhan and C.K. Shirkot (2013). Optimization of cultural conditions for cellulase-free xylanase production by mutant strain of alkalophilic *Cellulosimicrobium* sp. CKMX1 in submerged fermentation. *Appl. Biol. Res.,* **15:** 137-144.

Hui, L., C. Wan, D. Hai-tao, C. Xue-jiao, Z. Qi-fa and Z. Yu-hua (2010). Direct microbial conversion of wheat straw into lipid by a cellulolytic fungus of *Aspergillus oryzae* A-4 in solid-state fermentation. *Bioresour. Technol.,* **101:** 7556-7562.

Kapoor, M., L.M. Nair and R.C. Kuhad (2008). Cost-effective xylanase production from free and immobilized *Bacillus pumilus* strain MK001 and its application in saccharification of *Prosopis juliflora*. *Biochem. Eng. J*., **38:** 88-97.

Kavya, V. and T. Padmavathi (2009). Optimization of growth conditions for xylanase production by *Aspergillus niger* in solid state fermentation. *Polish Journal of Microbiology.*, **58(2):** 125-30.

Khan, A.W., D. Tremblay and A. Le Duy (1986). Assay of xylanase and xylosidase activities in bacterial and fungal cultures. *Enzyme Microb. Technol*., **8(6):** 373-377.

Miller, G.L. (1959). Use of dinitrosalicylic acid reagent for determination of reducing sugar. *Anal. Chem*., **31(3):** 426-428.

Okafor, U.A., V.I. Okochi, B.M. Onyegeme-okerenta and S. Nwodo-Chinedu (2007). Xylanase production by *Aspergillus niger* ANL 301 using agro – wastes. *Afr. J. Biotechnol*., **6(14):**1710-1714. [Google Scholar]

Pedersen, M.B., S. Dalsgaard, S. Arent, R. Lorentsen, K.E.B. Knudsen, S. Yu and H.N. Laerke (2015). Xylanase and protease increase solubilization of non-starch polysaccharides and nutrient release of corn- and wheat distillers dried grains with soluble. *Biochem. Engineering J.,* **98:** 99-106.

Polizeli, M.L.T.M., C.S. Rizzatti, R. Monti, H.F. Terenzi, J. Jorge and D.S. Amorim (2005). Xylanases from fungi: properties and industrial applications. *Applied Microbiology and Biotechnology.,* **67:** 577-91.

Muthezhilan, R., S. Jayalakshmi and R. Ashok (2007). Production and optimization of thermostable alkaline xylanase by *Penicillium oxalicum* in solid state fermentation, *African Journal of Microbiology Research*, **1(2):** 020-028, [Google Scholar]

Rahman, A.K., N. Sugitani, M. Hatsu and K. Takamizawa (2003). A role of xylanase, a-L-arabinofuranosidase and xylosidase in xylan degradation. *Can. J. Microbiol*., **49:** 58-64.

Sakthiselvan, P., B. Naveena amd N. Partha (2014). Molecular

characterization of a xylan producing fungus isolated from fouled soil. *Brazilian journal of Microbiology.,* **45(4):** 1293- 1302.

Shallom, D. and Y. Shoham (2003). Microbial hemicellulases. *Curr. Opinion Microbiol*., **6:** 219-228.

Singh, V., V.C. Pandey and S. Agrawal (2013). Potential of Laceyella sacchari strain B42 crude xylanase in biobleaching of kraft pulp. *Afr. J. Biotechnol.,* **12(6):** 570-579.

Singh, A., R.C. Kuhad and M. Kumar (1995). Xylanase production by a hyperxylanolytic mutant of *Fusarium oxysporum*. *Enzyme microb tech*., **17:** 551-553.

Thmoas, L., M.V. Ushasree and A. Pandey (2014). An alkali thermostable xylanase from *Bacillus pumilus* functionally expressed in *Kluyveromyces lactis* and evaluation of its deinking efficiency. *Biores. Technol.,* **65:** 309-313.

Tseng, M.T., M.N. Yap, K. Ratanakhanokchai, K.L. Kyu and S.T. Chen (2002). Purification and characterization of two cellulase free xylanases from an alkalophilic *Bacillus firmus. Enzyme Microbiol. Technol*., **30:** 590- 595.

Yadav, H. and A.K. Jaitly (2011). Effect of salt on the production of xylanase in some thermophilic fungi. *J. Phytol.,* **3:** 12-14.

Yaun, Q. and M. Rugyu (1999). Study on temperature oscillation in production of xylanase by *Aspergillus niger*. *Beijing Hugagong Daxue Xuebao.,* **26:** 11-16.

Zychlinski, A.W., J. Czakaj and S. Zukowska (1994). Xylanase production by fungal strains in solid state fermentation. *Bioresour. Technol*., **49:** 13-16.