African Journal of Biotechnology Vol. 6 (14), pp. 1710-1714, 18 July 2007
Available online at http://www.academicjournals.org/AJB
ISSN 1684–5315 © 2007 Academic Journals

Full Length Research Paper

Xylanase production by *Aspergillus niger* ANL 301 using agro - wastes

Okafor, U. A.[1], Okochi, V. I.[1,] Onyegeme-okerenta[1], B. M. and Nwodo-Chinedu, S.[2*]

[1]Department of Biochemistry, College of Medicine, University of Lagos, PMB 12003 Idiaraba, Lagos, Lagos State, Nigeria.
[2]Department of Biological Sciences, College of Science and Technology, Covenant University, KM 10 Idiroko Road, Canaan Land, PMB 1023 Ota, Ogun State, Nigeria.

Accepted 15 June, 2007

Xylanase production by wild-type *Aspergillus niger* ANL301, newly isolated from wood-waste, was monitored at 24 h intervals for a period 168 h in media containing different carbon sources. The carbon sources were oat-spelt xylan (Fluka) and three agro-wastes (sawdust, sugarcane pulp and wheat bran). Highest xylanase activity of 6.47 units/mL was obtained at 96 h in media containing wheat bran as sole carbon source. Maximum activity value for the media containing sugarcane pulp was 0.95 units/mL obtained also at 96 h. Sawdust and oat spelt xylan gave the peak enzyme activities of 0.65 and 0.80 units/mL respectively at 120 h. High protein yield was obtained in media containing the agro-wastes, with wheat bran giving the highest value of 1.14 mg/mL at 96 h. The maximum specific xylanase activities were 3.86, 3.37, 5.69, and 9.36 units/ mg protein for sawdust, sugarcane pulp, wheat bran and oat spelt xylan, respectively. Out of the three agro-wastes used in this study, wheat bran holds greatest promise for low cost production of the xylanase enzyme.

Key words: *Aspergillus niger* ANL301, agro-wastes, submerge fermentation, xylanase.

INTRODUCTION

Large quantities of lignocellulosic wastes are generated through forestry, agricultural practices and industrial processes, particularly from agro-allied industries such as breweries, paper-pulp, textile and timber industries. These wastes generally accumulate in the environment thereby causing pollution problem (Abu et al., 2000). Most of the wastes are disposed by burning, a practice considered as major factor in global warming (Levine, 1996). However, the plant biomass regarded as “wastes” are biodegradable and can be converted into valuable products such as biofuels, chemicals, cheap energy sources for fermentation, improved animal feeds and human nutrients (Howard et al., 2003).

Lignocelluloses are mainly secondary plant cell-wall materials which consist of lignin, cellulose and hemicelluloses (Grant and Long, 1981). D-xylan is the major hemicellulose found in woods and accounts for 20 - 35% of the total dry weight of hardwood and perennial plants (Haltrich et al., 1996). The basic structure of xylan is a β-D-(1, 4)-linked xylopyranosyl residue with a few branch points (Kulkarni et al., 1999). The major backbone carries relatively short side chains of variable lengths. Due to the abundance and the structural heterogeneity of xylans, xylan-degrading enzymes are diverse (Lee et al., 2003). Typical xylan-degrading enzymes are endo-β-xylanases (EC 3. 2. 1. 8) which attack the main chain of xylans, and β-xylosidases (EC 3. 2. 1. 37) which hydrolyze xylooligosaccharides into D-xylose. These two enzymes, also required for complete hydrolysis of native cellulose and biomass conversion, are produced by many bacteria and fungi. Potential applications of xylanase in biotechnology include bio-pulping of wood (Eriksson, 1985; Eriksson and Kirk, 1985), pulp bleaching (Jurasek and Paice, 1988; Kantelinen et al., 1988.), treating animal feed to increase digestibility (Wong et al., 1988), processing food to increase clarification (Biely, 1985), and converting lig-

*Corresponding author. E-mail: sncresearch@yahoo.com.

nocellulosic substances into feed stocks and fuels (Eriksson, 1985; Jeffries, 1985; Kim et al., 2000).

Filamentous fungi are attracting greater attention than bacteria as potential sources of plant cell wall hydrolyzing enzymes such as xylanases because they secrete high levels of the enzymes into the culture medium (Berry and Paterson, 1990). In our search for microorganisms capable of efficiently degrading lignocelluloses, some cellulolytic microfungi including a wild strain of *Aspergillus niger* (ANL301) were isolated from decomposing wood-wastes in Lagos, Nigeria (Nwodo-Chinedu et al., 2005). This microfungus grows effectively in mineral salt medium supplemented with sawdust or sugarcane as sole carbon sources (Nwodo-Chinedu et al., 2007). The present study was designed to investigate the potential use of some agro-wastes as carbon sources for xylanase production by the strain of *A. niger* ANL301. The aim is to evaluate the capacity of the agro-wastes to serve as low-cost substrates for xylanase production by the wild strain of *A. niger* (ANL301) via submerged fermentation.

MATERIALS AND METHODS

Chemicals

All chemicals and reagents were of analytical grade. Potato Dextrose agar and crystalline cellulose were obtained from Merck, Germany. Oat spelt xylan was from Fluka, Bioichemika, Germany. All other chemicals and reagents were obtained from Sigma Chemicals Company Limited, England.

Cellulosic materials

Sawdust of Abora wood (*Mitragyna ciliata*) was collected from Okobaba Saw-mills, Ebute-Metta, Lagos, Nigeria. Mature Sugarcane (*Saccharum offinarum*) and dry maize (*Zea mays*) were purchased from Oshodi market in Lagos, Nigeria. Fibrous pulp of the sugarcane was obtained by crushing and washing the pulp repeatedly in water to remove all residual sugars. Wheat bran was obtained at Mushin market in Lagos, Nigeria. The samples were dried in the oven at 80^{o}C for 2 h, ground with Marlex Exceller Grinder (Mumbai, India) and passed through a sieve (about 0.5 mm pore size) to obtain the respective fine powder used for the study.

Organism and growth studies

Isolate of *A. niger ANL301* (Nwodo-Chinedu et al., 2005) maintained at 4^{o}C on Potato Dextrose Agar (PDA) slants was sub-cultured on fresh sterile PDA plates and incubated for 72 – 120 h.

Media preparations and enzyme production

The media contained (per liter of distilled water): $NaNO_3$, 3.0 g; KCl, 0.5 g; KH_2PO_4, 1.0 g; $MnSO_4.7H_2O_4$, 0.5 g; $FeSO_4.7H_2O$, 0.01 g; and a carbon source (Sawdust, Sugarcane pulps, Wheat bran and Oat spelt xylan), 10.0 g. One liter (1 L) of the media was supplemented with 1.0 mL of trace solution containing (per liter of distilled water) $ZnSO_4$, 1.0 g and $CuSO_4.5H_2O$, 0.5 g. The pH of each media was adjusted to 5.6. Then, 100 mL of the respective liquid medium was placed in 250 mL Erlenmeyer flask and sterilize by autoclaving at 121°C for 15 min. This was cooled and inoculated with 10 discs of 5.0 mm diameter of the organism from PDA culture plates using a sterile cork borer. Cultures were harvested in triplicates at 24 h intervals by centrifugation at 1000 X g over a period of 168 h. The supernatants were the crude extracellular enzyme source.

Protein assay

Protein contents of the culture supernatants were assayed by the folin ciocalteau method of Lowry et al. (1951) using Bovine Serum Albumin (BSA) as standard.

Xylanase assay

A modification of the reducing sugar method described by Khan (1980) was used for the assay of xylanase (EC 3. 2. 1. 8) activity. Oat spelt xylan (Fluka) was used as enzyme substrate. The reaction mixture contained 0.5 mL of 0.1% (w/v) substrate in 0.1 M sodium acetate buffer (pH 5.0) and 0.1 mL of cell-free culture supernatant. The mixture was incubated at 40^{o}C in water bath with shaking for 30 min. The reducing sugar released was measured using 3,5-dinitrosalicylic acid (Miller, 1952) and xylose as standard. The colour was developed by boiling in water bath for 5 min. Absorbance was read at 540 nm using spectrophotometer (Spectronic Genesys TMS, USA). The released reducing sugar was expressed in units mL^{-1}. One unit of activity was defined as amount of enzyme required to liberate 1 µmol of xylose per minute under the assay conditions. The xylanase activities of *A. niger* ANL 301 cultivated on agro-wastes (sawdust, sugar pulp, and wheat bran) and oat spelt xylan was monitored at 24-h intervals for a period of 168 h under submerged fermentation condition.

RESULTS

Figure 1 shows the xylanase activity of the culture supernatant of *A. niger* ANL301 from the different media. Xylanase activities of the culture supernatants from the different media increased as incubation progressed to a peak value after which they declined. A very high xylanase activity peak of 6.47 units/mL was obtained at 96 h for the culture supernatant from media containing wheat bran. This is about 7 times the maximum activity obtained with any of the other carbon sources. The media containing sugarcane pulp gave a peak xylanase activity value of 0.95 units/mL at 96 h. Media containing sawdust and oat spelt xylan showed two activity peaks, the major peak at 120 h and a minor peak at 72 h, and a depressed activity in-between them at 96 h. The maximum xylanase activities in media containing sawdust and xylan were 0.68 and 0.80 units/ mL, respectively.

The protein released by *A. niger* ANL 301 in the media containing the respective carbon sources is shown in Figure 2. Cultures containing the agro-wastes gave higher protein levels compared to that containing oat spelt xylan. The highest value was obtained with wheat bran cultures which gave a maximum protein concentration of 1.14 mg/mL at 96 h. Protein peaks of 0.68 and 0.50 mg/ mL were obtained respectively from cultures containing sawdust and sugarcane pulp at 144 h. The least protein peak value of 0.38 mg/ mL was obtained

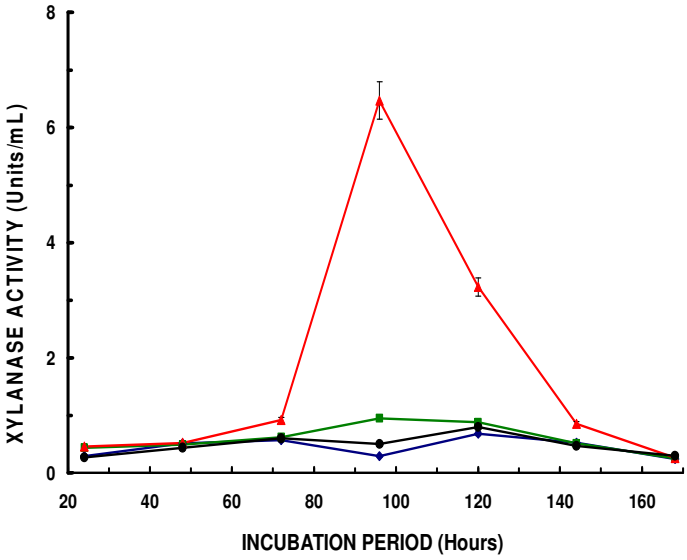

Figure 1. Xylanase activity of *Aspergillus niger* ANL301 cultured at 30ºC in modified Czapek-Dox broth containing sawdust (-♦-), sugarcane pulp (-■-), wheat bran (-▲-) and oat spelt xylan (-●-) as sole carbon sources. (1 Unit of xylanase activity = 1 µmol xylose min $^{-1}$)

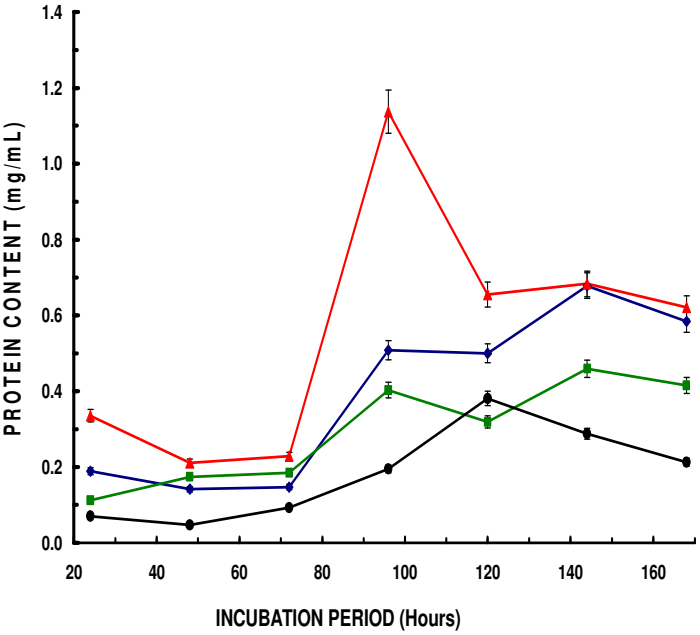

Figure 2: Protein content of culture supernatant of *Aspergillus niger* ANL301 incubated at 30ºC in modified Czapek-Dox broth containing sawdust (-♦-), sugarcane pulp (-■-), wheat bran (-▲-) and oat spelt xylan (-●-) as sole carbon sources.

from cultures containing xylan at 120 h.

Figure 3 shows the specific xylanase activities of *A. niger* ANL301 cultured in the different carbon sources. The highest specific xylanase activity value of 9.36 units/mg protein was obtained at 48 h from the culture containing oat spelt xylan. Maximum specific activities of 3.86 and 3.37 units/mg protein were obtained at 72 h for cultures containing sawdust and sugarcane pulp respectively. The maximum specific activity from the culture containing wheat bran was 5.69 Units/ mg Protein. This

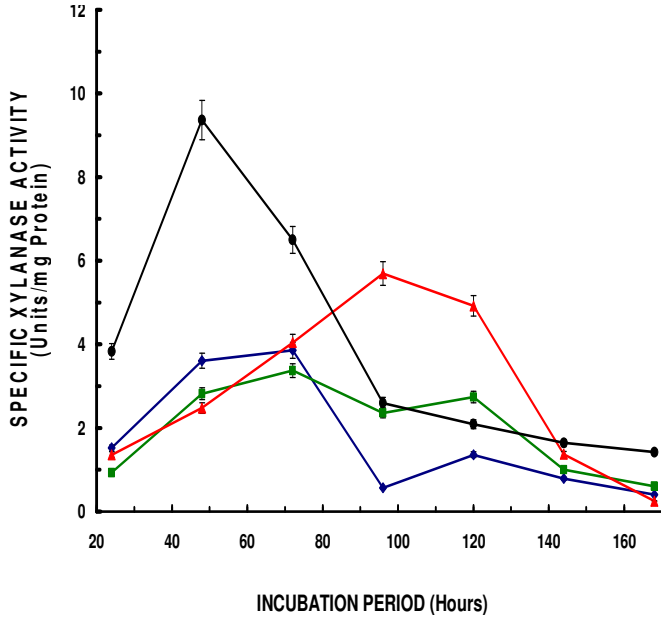

Figure 3. Specific xylanase activity of *Aspergillus niger* ANL301 cultured at 30ºC in modified Czapek-Dox broth containing sawdust (-♦-), sugarcane pulp (-■-), wheat bran (-▲-) and oat spelt xylan (-●-) as sole carbon sources. (1 Unit of xylanase activity = 1 µmol Xylose min $^{-1}$)

was obtained at 96 h of incubation.

DISCUSSION

The results show that *A. niger* ANL 301 produces xylanase enzyme (EC 3. 2. 1. 8) when cultured in media containing the different agro-wastes (sawdust, sugarcane pulp and wheat bran) as sole carbon sources. Extracellular proteins with significant xylanase activity were obtained from the cultures of all the different carbon sources (Figures 1 and 2). Most members of the *A. niger* group are notable producers of extracellular enzymes including important plant cell-wall hydrolyzing enzymes such as xylanases (de Vries and Visser, 2001). The protein levels and xylanase activities of the crude enzyme preparations from the different carbon sources however differed significantly. The highest level of xylanase activity was obtained with wheat bran. The level of protein secreted by the organism in media containing wheat bran was also the highest compared to the media containing any of the other carbon sources (Figure 2). The lowest protein level was found in media containing xylan. This is the basis for the high specific xylanase activity obtained in xylan containing media (Figure 3). The organism gave the highest specific xylanase activity value in xylan-containing media. In spite of the high protein level of the media containing wheat bran, the maximum specific xylanase activity obtained with the substrate was very close to that obtained with pure xylan. The period of optimum xylanase activity also varied with the different carbon sources. The time was shorter for wheat bran and sugarcane pulp (96 h) than for oat spelt xylan and sawdust (120 h). This shows that of the three agro-wastes, wheat bran is the best prospective carbon source for the production of the enzyme.

High cost of production of plant cell-wall hydrolyzing enzymes is a limiting factor in their commercial production and industrial applications (Spano et al., 1978). One area currently considered as cost-reduction strategy is the use of waste plant materials as carbon sources for the production of the enzymes. In a recent research carried out by Villas-Boas and his colleagues (2002), xylanase production by *Candida utilis* using apple pomace was found to be comparatively low. In another study, xylanase was produced from *Pleurotus* sp. using banana (agricultural) wastes (Reddy et al., 2003). In a similar research carried out with different agro-wastes (orange pomace, orange-peel, lemon pomace, lemon peel, apple pomace, pear peel, banana peel, melon peel and hazelnut shell), melon peel followed by apple pomace and hazelnut shell gave the best xylanase activity (Seyis and Aksoz, 2005). Some other fungal species had also shown promising results in terms of xylanase yield with different agro-wastes (Atev et al., 1987; Kadowaki and Souza, 1997).

Our data has shown that the wild-type *A. niger* ANL301 can produce extracellular proteins with significant xylanase activity when cultivated in media containing different agro-wastes as sole carbon sources. It also reveals that

wheat bran, compared to the other agro-waste materials studied, is a very promising substrate for xylanase production. The use of agro-wastes in the production of such enzymes as xylanases will ultimately bring down their production cost and at the same time reduce environmental pollution due to the wastes.

REFERENCES

Abu EA, Onyenekwe PC, Ameh DA, Agbaji AS, Ado SA (2000). Cellulase (E. C. 3. 2. 1. 3) production from sorghum bran by *Aspergillus niger* SL 1: An assessment of pretreatment methods. Proceedings of the International Conference on Biotechnology: Commercialization and Food security, Abuja, Nigeria. pp. 153–159.

Atev AP, Panoyotov CA, Bobareva LG, Damyanova LD, Nicolava D (1987). Studies of biosynthesis of hydrolysis by *Trichoderma sp* m7 on submerged and solid-state cultivation conditions. Acta Biotechnologica, 7: 9-16

Biely P (1985). Microbial xylanolytic system. Trends Biotechnol. 3: 286-291.

Eriksson KEL (1985). Swedish developments in biotechnology related to the pulp and paper industry. Tech. Assoc. Pulp Pap. Ind. 68: 46-55.

Eriksson K-EL, Kirk TK (1985). Biopulping, biobleaching and treatment of kraft bleaching effluents with white-rot fungi. In C. W. Robinson (ed), Comprehensive biotechnology. Pergamon Press, Toronto. pp. 271-294.

Haltrich D, Nidetzky B, Kulbe KD, Steiner W, Zupancic S (1996). Production of fungal xylanases. Bioresource Tech. 58: 137-161.

Howard RL1, Abotsi E, Jansen van Rensburg EL1, Howard S (2003.) Lignocellulose biotechnology: issues of bioconversion and enzyme production. Review. Afr. J. Biotechnol. 2(12): 602-619.

Jeffries TW (1985). Emerging technology for fermenting D-xylose. Trends Biotechnol. 3: 208-212.

Jurasek L, Paice MG (1988). Biological bleaching of pulp. In International pulp bleaching conference. Technical Association of the Pulp and Paper Industry, Atlanta, pp. 11-13.

Kadowaki MK, Souza CGM (1997). Xylanase production by *Aspergillus tamarii*. Appl. Biochem. Biotechnol. 66: 97-106.

Kantelinen A, Ratto M, Sundqvist J, Ranua M, Viikari L, Linko M (1988). Hemicellulases and their potential role in bleaching. Proceeding of International Pulp Bleaching Conference, Tappi, Atlanta, 1998. pp. 1-9.

Kim JH, Kim SC, Nam SW (2000). Constitutive overexpression of the endoxylanase gene in *Bacillus subtilis*. J. Microbiol. Biotechnol. 10: 551-553.

Kulkarni N, Shendye A, Rao M (1999). Molecular and biotechnological aspects of xylanases. FEMS Microbiol. Rev. 23: 411-456.

Lee RC, Hrmova M, Burton RA (2003). Bifunctional family 3 glycoside hydrolases from Barley with alpha-L-Arabinofuranosidase and beta-D-Xyylosidase activity characterization, primary structure and COOH-terminal processing. J. Biol. Chem. 278: 5377-5388.

Levine JS (1996). Biomass burning and global change. In: Levine JS (eds.) (vol. 1) Remote sensing and inventory development and biomass burning in Africa. The MIT Press, Cambridge, Massachusetts, USA. p. 35.

Lowry OH, Rosebrough NJ, Farr AL, Randall RJ (1951). Protein measurement with the folin-phenol reagent. J.Biol. Chem. 193: 265-275.

Miller GL (1959). Use of dinitrosalicyclic reagent for the determination of reducing sugars. Anal. Chem. 31: 426-428.

Nwodo-Chinedu S, Okochi VI, Smith HA, Omidiji 0 (2005) Isolation of cellulolytic microfungi involved in wood-waste decomposition: Prospect for enzymatic hydrolysis of cellulosic wastes. Int. J. Biomed. Health Sci. 1(2): 41-51.

Nwodo-Chinedu S, Okochi VI, Omidiji O, Omowaye OO, Adeniji BR, Olukoju D, Chidozie F (2007). Potentials of cellulosic wastes in media formulation. Afr. J. Biotechnol. 6(3): 243-246.

Reddy GV, Babu PR, Komaraih P, Roy KRRM, Kothari IL (2003). Utilization of banana waste for the production of lignolytic and cellulolytic enzymes by solid substrate fermentation using two *Pleurotus* species (*P. ostreatus* and *P. sajorcaju*), Process Biochem. 381. 457–1462.

Seyis I, Aksoz N (2005). Xylanase Production from *Trichoderma harzianum*. Food Technol. Biotechnol. 43(1): 37–40.

Spano L, Alien A, Tarssinane T, Mandels M, Ryu DD (1978). Reassessment of economics of Technology for production of ethanol Proceedings in Industrial fuel from biomass symposium. Troy, New York. pp. 671-674.

Villas-Boas SG, Esposito E, de Mendonca MM (2002).Novel lignocellulolytic ability of *Candida utilis* during solid-substrate cultivation on apple pomace, World J. Microbiol. Biotechnol. 18: 541–545.

Wong KKY, Tan LUL, Saddler JN (1988). Multiplicity of α-1, 4 xylanase in microorganisms' functions and applications. Microbiol. Rev. 52: 305-317.