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Xylanase-production potential of *Trichoderma Asperellum* NG-T161 and NG-T163 isolated from banana farm soils in South Western Nigeria

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Abstract

The detrimental effect associated with the use of chemicals in industries and wastes treatment of lignocellulosic origin has led to an increase in the demand for xylanase. Hence, there is need for continuous search for more microorganisms with ability to produce this important enzyme. This work is aimed at producing and assaying xylanase using stains of *Trichoderma asperellum* which is abundant in banana soil. Species of *Trichoderma asperellum* (NG-T163) and (NG-T161) isolated from banana soil in Ede and Gbogan were screened for production of xylanase. The influence of xylan concentrations, carbon and nitrogen sources and pH were evaluated with respect to xylanase production. Production of the enzyme, using lignocellulosic materials by the fungi, was also investigated. The enzyme was partially purified and applied to degrade agro-industrial wastes. The highest xylanase production was obtained on yeast extract having activity of 1.5 mg/mL. Enzyme activity was highest at 2% xylan with 3.53 mg/mL for NG-T163. Lactose and sucrose were the best carbon sources for the induction of the enzyme. Optimum xylanase production was observed at a pH of 5.0 for NG-T161and 6.0 for NG-T163. Lignocellulosic wastes induced production of xylanase; the highest enzyme activity (4.0 mg/mL) was obtained with purified xylanase in the presence of banana peels by the two isolates. It can therefore be deduced that *Trichoderma asperellum* (NG-T163 and NG-T161) have the ability to produce good quantum of xylanase and good prospectus source of xylanase for industrial use and in the treatment of plant wastes.

Key words – Bagasse – Enzyme – Lactose – Microorganisms – Wastes

Introduction

Xylanases are a group of xylan-degrading enzymes (linear polysaccharide β-1, 4-xylan) present in hemicellulose. Although most microorganisms are reported to produce these enzymes, filamentous fungi are the major commercial source of xylanases (Haltrich et al. 1996). Hemicellulose is a group of noncellulosic polysaccharides which consist of xylan, xyloglucan (α-heteropolymer of D-xylose and D-glucose), glucomannan (α- heteropolymer of D-mannose and D-glucose), galactoglucomannan (α- heteropolymer of D-galactose, D-glucose and D-mannose),

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arabinogalactans (α-heteropolymer of Dgalactose and arabinose) (Shallom & Shoham 2003). Xylanases are also called endo-1,4-β-xylanase, but other synonyms include xylanase, endoxylanase, β-1,4-D-xylan- xylanohydrolase, endo-1,4-β-D-xylanase, β-1,4- xylanase and β-xylanase (Chundawat et al. 2011). Among xylanolytic enzymes, endoxylanase and β-xylosidase are the key enzymes responsible for xylan hydrolysis. These enzymes are characterized by their potential to breakdown various xylans to produce short chain xylo oligosaccharides, depending on the length of the chain, degree of branching and the presence of substituents in the substrate molecule (Li et al. 2000). These enzymes readily crystallize in ammonium sulphate and phosphate of sodium/ potassium at a pH range of 3.5-9 and within temperature range of 10-37°C (Alebiosu et al. 2015).

Agricultural wastes compose of essential cellulosic or lignocellulosic residues which are considered to be the cheapest source for the production of different valuable products. In addition, agro-industrial wastes can be used as source of fermentable sugars and ethanol production (Chandel et al. 2012, Anwar et al. 2014). Xylan, which is the second most abundant polysaccharide in nature after cellulose, is part of the hemicellulose present in the amorphous matrix of the secondary cell wall of lignified tissue of woody plants, cereals and other plant materials (Beg et al. 2001, Polizeli et al. 2005, Collins et al. 2006, Motta et al. 2013). Various species of fungi have been implicated in xylanase production (Maria et al. 2002, Sridevi & Charya, 2011, Namasivayam et al. 2015).

The wide spread application of microbial xylanases in industrial processes and the detrimental effect associated with the use of chemicals in industries has led to an increase in the demand for xylanase. This enzyme has outstandingly wide range of potential biotechnological applications in pulp and paper industry, production of xylo-oligosaccharides, texture improvement of bakery products, textile industry, nutritional improvement of pig/poultry feed, fruit softening/clarification of juices and wines, bioconversion of lignocellulosic material and agro-wastes to fuels and chemical feedstock, production of pharmaceutically active polysaccharides for use as antimicrobial agents or antioxidants, detergents, extraction of coffee and plant oils and pigments, and degumming of plant fibers such as flax, hemp and jute (Wong et al. 1988). Various optimized culture conditions required for the xylanase production coupled with lignocellulosic substrate as carbon source will provide a reliable platform for effective and fast means of xylanase production; the use of this enzyme to replace chemical use in the industry will contribute to the reduction in environmental pollution associated with the use of chemicals. In other to meet up with this demand, there is a need to find means for producing a cost effective xylanase on a large scale. This study is therefore aimed to screen for the xylanase producing ability of *Trichoderma asperellum* isolated from soil and to investigate the production of this enzyme using various lignocellulosic substrates which constitute environmental pollution.

This research was carried between February and November 2017 at Federal University Oye-Ekiti, Nigeria

Materials & Methods

Trichoderma asperellum strains used in this study were earlier isolated from banana farm soil in South Western Nigeria and identified at the Plant Science Institute, Systematic Botany and Mycology Laboratory, USDA Agricultural Service, Baltimore Avenue, Beltsville MD 20705-2350. (Adebesin et al. 2005).

Screening of fungal isolates for xylanase production

*Trichoderma asperellum*NG-T163 and NG-T161 were screened for their ability to produce extracellular xynalase using modified mineral solution described by Hedger & Hudson (1974). ($NaNO_3$,0.5% KH_2PO_4, 0.136% $MgSO_4.7H_2O$, 0.05% $Ca(NO_3)_2$, 0.001% $FeCl_3$ 0.0001% wood xylan 1%, tryptone 0.1%, yeast extract 0.05%, K_2HPO_4 0.174% and agar 1.2%). The fungi were incubated at 25°C for five days, after which the zone of hydrolysis around the fungal colonies was observed by flooding with 0.1% congo red for 20 minutes, followed by subsequent washing with

1M NaCl for 20mins and later with 1N HCl for another 20mins for more clarity of the zone of hydrolysis.

Effect of varying concenterations of xylan on the synthesis of xylanase by *Trichoderma asperellum*.

The effect of different concentrations of xylan on xynalase production was studied by varying the concentration of xylan from 0.5 %, 1.0 %, 1.5 %, 2.0 % and 2.5 % w/v. 10 mL of each of the concentrations was inoculated with 2 ml of spore suspension of 5 days old fungus and incubated for five days before filtration and the filtrate was used as source of crude enzyme.

Enzyme Assay

Xynalase activities were determined by mixing 0.5 mL of 1% w/v xylan in 0.1M citrate buffer pH 5.0 with 0.5 mL of crude enzyme and the mixture was incubated at 50°C for 30 mins. The reaction was stopped by addition of 1 mL 3, 5 dinitrosalicylic acid and the content was boiled for 5 minutes inside water bath at 100°C according to (Miller 1959) and cooled immediately. The color developed was read at 540 nm using spectrophotometer.

Xylose was used as standard and xylanase activity was expressed as 1mg of reducing sugar (xylose equivalent) per milliliter of enzyme solution.

Effect of different carbon sources on enzyme production strains of *Trichoderma asperellum*

Effect of different carbon sources on xylanase production was studied by growing the fungi in different carbon sources including glucose, galactose, maltose, fructose, lactose and sucrose. Each of the sugar was used to replace xylan in modified Hedger and Hudson broth medium. The medium was autoclaved at 121°C for 15 minutes and allowed to cool. 2 mL of the dislodged spores was inoculated into 10 mL of carbon broth, incubated for 5 days and filtered to obtain crude enzyme. Xylanase activity was assayed as previously described.

Effect of different nitrogen sources on xylanase synthesis by strains of *Trichoderma asperellum*

Effect of different nitrogen sources in xylanase production was carried out using both organic and inorganic sources; urea, tryptone, yeast extract, ammonium nitrate (NH_4NO_3) and peptone. Each of these nitrogen sources was added to the broth medium in different tubes and sterilized. Each of medium was inoculated with 2 mL of the dislodged spores of 5days old culture incubated for five days and filtered. Xylanase activity was determined as described earlier.

Effect of varying pH values on the synthesis xylanase by strains of *Trichoderma asperellum*.

The effect of initial pH on enzyme production were determined at different pH values ranging from 4.0 to 7.0. Citrate phosphate buffer was used to prepare the medium and the medium was inoculated with 2 mL of the dislodged sporesand incubated for 5 days at 30°C before filtration. Xynalase activity was assyed by mixing 0.5 mL of the filtrate with 0.5 mL of 1 %w/v xylan and incubated at 50°C for 30 minutes after which the reaction was stopped by addition of 1 mL DNSA and boiled for 5 minutes at 100°C inside water then cooled immediately. The color change was read at 540nm using spectrophotometer

Preparation of inoculum for hydrolysis of lignocellulosic substrates

This was done by combining the optimized conditions for the two strains of the fungi. The modified medium for NG-T163 consisted lactose, yeast extract and K_2HPO_4 while it was glucose, yeast extract and K_2HPO_4 for NG-T161. The media were constituted, sterilized at 121°C for 15 minutes and allowed to cool. The media were inoculated with *Trichoderma* sp. NG-T163 and NG-T161 as appropriate in each flask and incubated for 96 hours at 25°C. The growth media were filtered and 5mL from each of the filtrate was used to degrade the lignocellulosic substrates. Lignocellulosic substrates; corn cob, groundnut shell, saw dust, and sugarcane bagasse were

collected from Ayegbaju and Oye in Ekiti state, Nigeria, and then transported to the laboratory for the study. The crude enzyme was the partially purified with ammonium sulphate ($(NH_4)_2SO_4$) precipitation within the limit of 0% and 100% saturation (Dixon & Webb 1979). The precipitate was re-suspended to initial volume of the crude enzyme filtrate with 50mM acetate buffer pH 5.0 and then dialyzed with the same buffer.

Pretreatment of lignocellulosic substrates

The substrates were alkaline treated by autoclaving the washed and dried substrate at 121ºC for 30 mintues with 0.25 M of NaOH (20 mL/g substrate). The substrate was recovered by filtration through muslin cloth, thoroughly washed with deionized water and neutralized with 0.25 M of HCl. The substrates were finally washed with many changes of deionized water and dried at 65ºC in the oven to constant weight (Singh & Kumar 1998).

Hydrolysis of substrate

A suspension of substrate (10 mg/mL) was prepared by adding 100mL of 50 mM citric phosphate buffer to 1 g of the substrate. 15 mL each of the substrate suspension was sterilized at 121ºC for 20minutes in 250 ml conical flask. 5 mL of crude enzyme was added to the substrate. Hydrolysis was performed at 30ºC for 5days and samples were withdrawn at 1hr, 24 hr, 48 hr, 72 hr and 96 hr for analysis of reducing sugar produced. The resultant filtrate following filtration was assayed for total releasing sugar using DNSA method (Miller 1959). The released sugar is expressed as equivalent to xylose. Xynalase activity was assayed by mixing 0.5 mL of the filtrate with 0.5 mL of 1 % xylan and incubated at 50ºC for 30 minutes after which the reaction was stopped by addition of 1 mL DNSA and boiled for 5 minutes at 100ºC inside water then cooled immediately. The color change was read at 540nm using (T 60 UV/Visibe spectrophotometer)

The purified xylanase was also used in the biodegradation of treated corn cob, banana peel and plantain peel.

Results

The two strains (NG-T163) and (NG-T161) of the fungi showed zone of hydrolysis (2.5 cm) and (1.4 cm) respectively on xylan agar plates. Based on the hydrolysis on the plate, the quantitative enzyme production was studied in the presence of different xylan concentrations, pHs, carbon sources, nitrogen sources and periods of incubations in lignocellulosic materials.

Effect of different concentrations of xylan, different carbon and nitrogen sources, initial pHs, growth periods on lignocellulosic wastes on xylanase production by the strains of *Trichoderma asperellum* as well as degradation of lignocellulosic wastes by purified enzyme are shown in Figs 1, 2, 3, 4, 5, 6.

The two strains (NG-T163) and (NG-T161) of the fungi showed zone of hydrolysis (2.5 cm) and (1.4 cm) respectively on xylan agar plates. Based on the hydrolysis on the plate, the quantitative enzyme production was studied in the presence of different xylan concentrations, pHs, carbon sources, nitogen sources and periods of incubations in lignocellulosic materials.

Effect of different concentrations of xylan on xylanase production by the strains of *Trichoderma asperellum*.

Enzyme synthesis increased as the concentration of xylan increased (Fig. 1) up to 2.0 % (3.6 mg/mL) for (NG-T161) after which there was no further increase. While in the case of (NG-T163), the maximum xylanase production was observed at 2.5 % concentration of xylan (3.64 mg/mL). The least values of enzyme production for the two strains are 1.45mg/mL and 2.4mg/mL for (NG-T161) and (NG-T163) respectively at 0.5 %.

Effect of carbon sources on xylanase production by the strains of *Trichoderma asperellum*.

The highest (5.34 mg/mL) and lowest (2.6 mg/mL) xylanase activity was observed in lactose and fructose culture (respectively) for isolate (NG-T163). Moreover, sucrose culture of isolate

(NG-T161) recorded the highest (5.88 mg/mL) xylanase activity, followed by maltose (4.86 mg/mL) (Fig. 2). However, the minimum enzyme production of 3.4 mg/mL for (NG-T161) was obtained in the medium containing galactose.

Effect of nitrogen sources on xylanase production by the strains of *Trichoderma asperellum*.

Enzyme production by isolate NG-T163 in different nitrogen sources ranged from 1.5 mg/mL, 1.13 mg/mL, 1.11 mg/mL, 0.9 mg/mL, to 0.5 mg/mL in yeast extract, peptone, tryptone, urea and NH_4NO_3 in that order while that of isolate NG-T161 ranged from 1.16 mg/mL, 1.14 mg/mL, 1.0 mg/mL, 0.8 mg/mL, to 0.6 mg/mL in tryptone, peptone, yeast extract, NH_4NO_3 to, urea respectively (Fig. 3).

Effect of pH on xylanase production by the strains of *Trichoderma asperellum*.

In Fig. 4, xylanase production by the fungi were maximum at a slightly acidic pH range of 5 to 6; increased pH above the range declined the enzyme productivity by the organisms. The xylanase production increased gradually for the two fungi as the pH increased from 3.0 to 5.0, at which point the production began to decrease for NG- T161. However, there was increment in enzyme production in the case of NG-T163 up to pH 6.0. The highest xylanase activity (3.04mg/mL) for NG- T161 was at pH 5.0 while that of NG-T163 (3.8mg/mL) was at pH 6.0. The least enzyme activities for the two strains are 1.2mg/mL and 1.04mg/mL for NG- T161 and NG-T163 respectively at pH 7.0.

Effect of incubation period on xylanase production in lignocellulosic wastes by the strains of *Trichoderma asperellum*.

The ability of the two strains of fungi to produce xylanase in the presence of different lignocellulosic wastes indicates that each of the substrates elicited different susceptibility to the utilization by the fungi at various periods. NG-T163 showed gradual increase in the amount of xylanase produced in sawdust (SD) from 24 h to 48 h, after which there was decrease of enzyme produced at 72 h and another increase at 96 h. In the medium containing sugarcane bagasse (SB), highest enzyme production was at 24 h which then decreased at 48 h and again increased at 96 h of incubation. Highest enzyme activity (1.84 mg/mL) was produced at 72 h in the medium with corncob (CC) after which the production decreased to (1.41 mg/mL) at 96 h. Similar observation was noted for groundnut shell (GS) at 72 h where the highest xylanase (2.16 mg/mL) was recorded after which there the production of the xylanase decreased to 1.8 mg/mL at 96 h.

For NG-T161, in SD, the highest amount of enzyme was produced at 24 h (1.5 mg/mL) after which the production of enzyme increased progressively to (0.44 mg/mL) at 72 h and at 92 h, the enzyme production increased (1.41 mg/mL). In SB, the highest value of zylanase (1.92 mg/mL) was recorded at 96 h and the least (0.92) was observed at 72 h. There was slight increase between 24 h and 48 h. In the medium containing CC, the highest amount of the enzyme (2.24 mg/ mL) was obtained at 48 h after which the value decreased at 72 h (1.36 mg/mL) and this followed by increase in the amount of zylanase produced (1.76 mg/mL) at 96 h. The highest enzyme production in GS was 1.92 mg/ mL at 96 h while the least amount (0.68 mg/mL) was obtained at 48 h. Increment in the zylanase production was observed between 72 h and 96 h.

Time course degradation of lignocellulosic wastes with purified xylanase produced by the strains of *Trichoderma asperellum*

There was gradual increment in the hydrolytic ability/time of the purified enzyme in all the wastes in this study (Fig. 6). Enzyme activity for Plantain peel increased from 0.36 to 3.6 mg/mL with purified xylanase NG-T 161 from 24 h to 96 h while it was 0.07 to 2.68 mg/mL for NG-T 163 for the same period. For banana peel, 0.14mg/mL of activity, as the lowest value was obtained at 24 h for the two isolates and the highest values of 4.0 mg/mL were obtained at 96 h of fermentation. Apart from banana, where NG-T163 had the highest value of 3.48 mg/mL at 72 h, NG-T161 gave the highest values in all the substrates and at all hours.

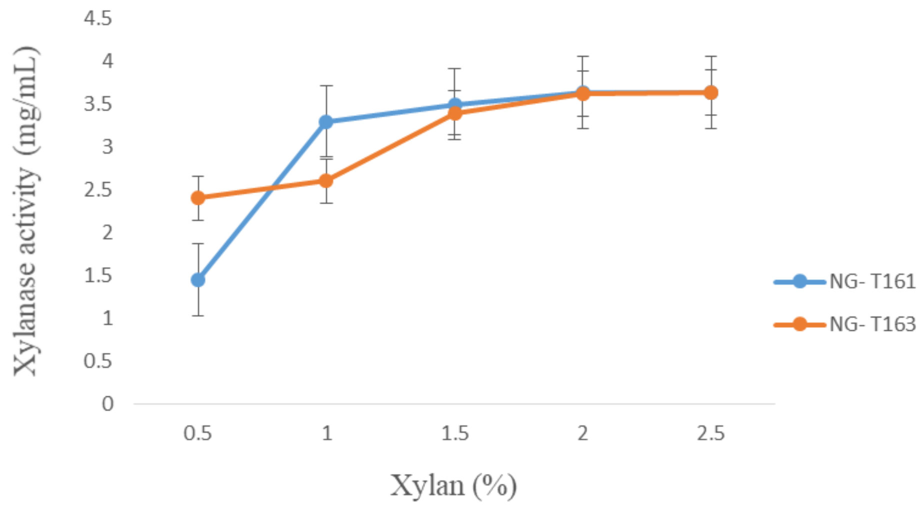

Fig 1 – Effect of different concentrations of xylan on the production of xylanase by strains of *Trichoderma asperellum*

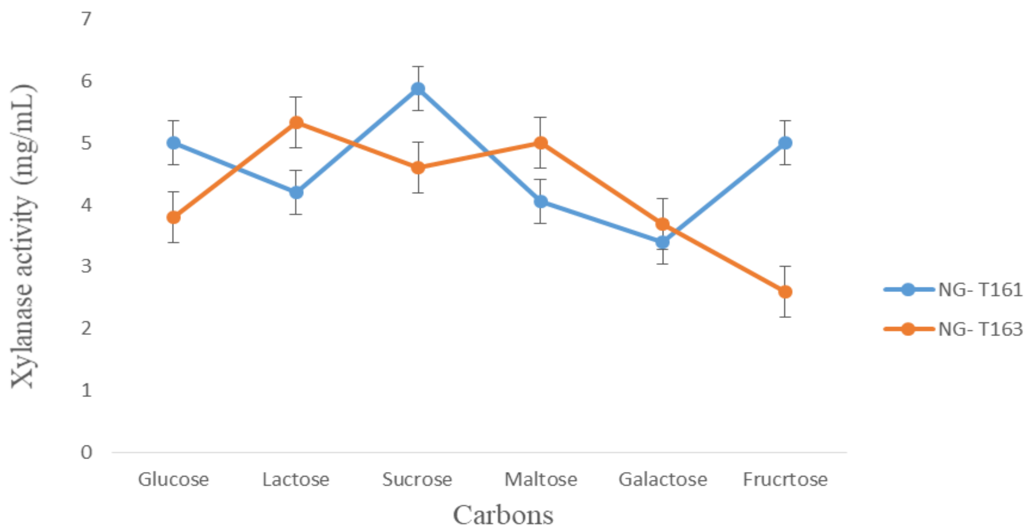

Fig. 2 – Effect of different carbon sources on the production of xylanase by strains of *Trichoderma asperellum*

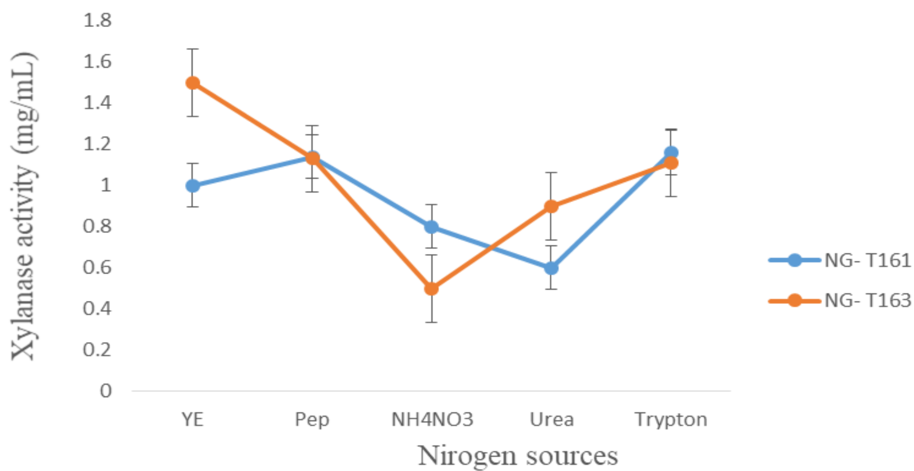

Fig. 3 – Effect of different nitrogen sources on the production of xylanase by strains of *Trichoderma asperellum*

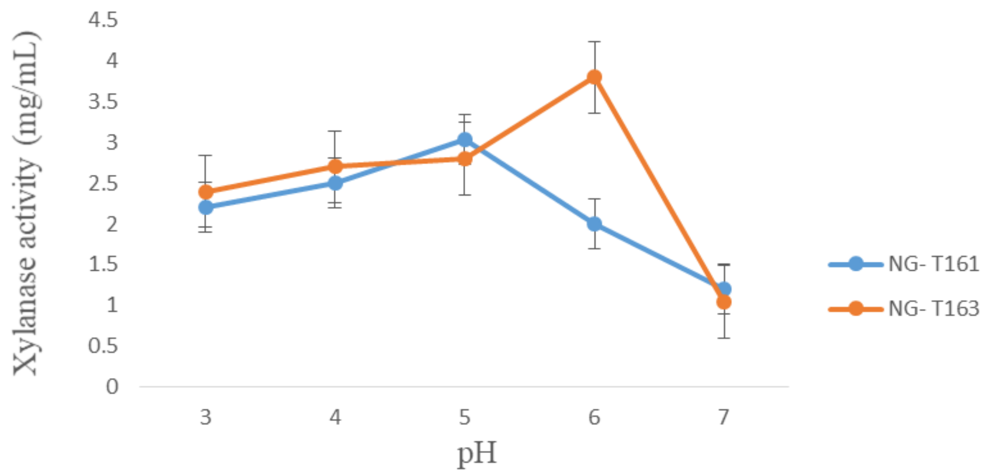

Fig. 4 – Effect of different pHs on the production of xylanase by strains of *Trichoderma asperellum*

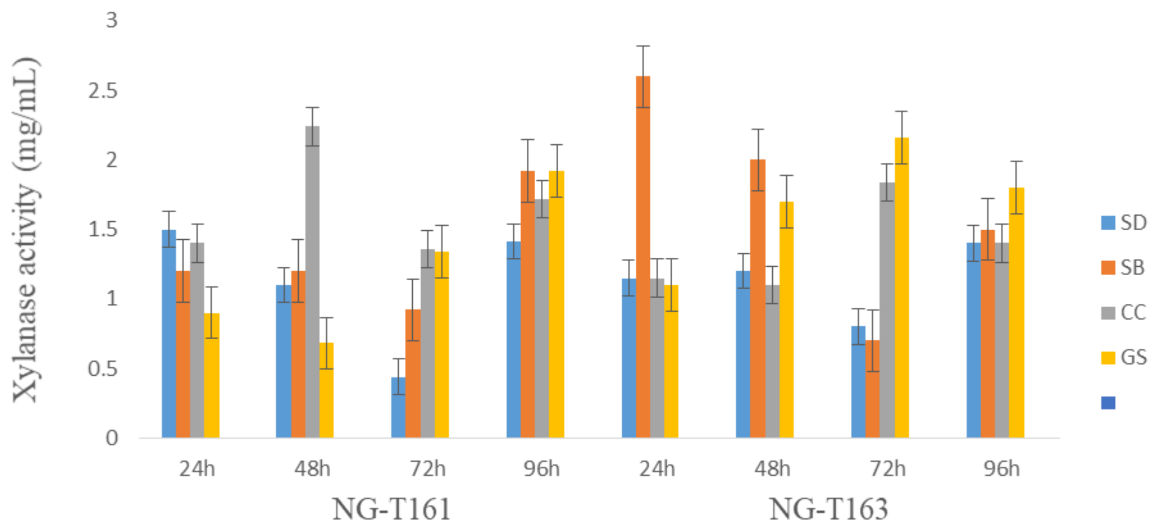

Fig. 5 – Effect of different growth periods on lignocelluloise wastes on the production of xylanase by strains of *Trichoderma asperellum.*

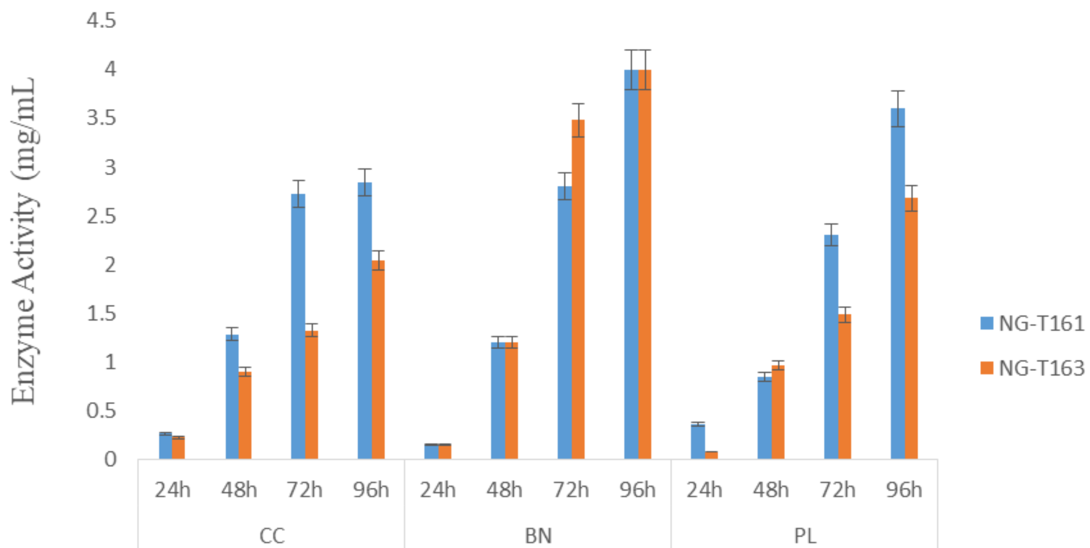

Fig. 6 – Effect of purified xylanase of *Trichoderma asperellum* strains on the hydrolysis of lignocellulosic wastes. CC = Corn cob, BN = Banana peel, PL = Plantain peel.

Discussion

Production of enzyme by *Trichoderma* sp. has been reported in few previous studies (Muhammad & Quratulain 2012). Ajijolakewu et al. (2015) had also reported the production of xylan degrading potential of some fugal species. Enzyme synthesis by the fungi increased as the concentration of xylan increased (Fig. 1). For isolate NG-T163, there is a gradual increase in the xylanase activity (2.4 mg/mL) at 0.5 % to 3.0 mg/mL at 1%. Gradual increment was also observed between 1% to 1.5 % xylan. The increment is in contrast when compared to isolate NG-T161, which has sharp increment of enzyme production between 0.5 % with enzyme activity of 1.46 mg/mL to 1% with enzyme activity of 3.50 mg/mL. The observed difference might not be unconnected with different species or strain involved in the work.

Effect of carbon sources on xylanase production by the strains of *Trichoderma asperellum*

The quantitative xylanase activity of enzyme produced by the isolates while grown on the broth having glucose, sucrose, lactose, fructose, and galactose as a carbon source shows (Fig. 2) that isolate NG-T163 produced maximum xylanase activity on lactose having enzyme activity of (5.34mg/ml) while fructose shows the lowest xylanase activity of 2.6mg/ml. In the case of isolate NG-T161 the highest enzyme activity was observed on sucrose with xylanase activity of 5.88mg/ml. while the lowest xylanase activity was observed on galactose having enzyme activity of 3.34mg/ml. Saleem et al. (2002), reported that the use of sucrose as a carbon source for xylanase production yield higher enzyme activity due to easy absorption of sucrose by microorganism. Production of substantial amount of xylanase had also been reported for *Trichoderma* spp by supplementing with sucrose (Pang et al. 2006).

Effect of nitrogen sources on xylanase production by the strains of *Trichoderma asperellum*.

Enzyme production by isolate NG-T163 in different nitrogen sources ranged from 1.5 mg/mL, 1.13 mg/mL, 1.11 mg/mL, 0.9 mg/mL, to 0.5 mg/mL in yeast extract, peptone, tryptone, urea and NH_4NO_3 in that order while that of isolate NG-T161 ranged from 1.16 mg/mL, 1.14 mg/mL, 1.0 mg/mL, 0.8 mg/mL, to 0.6 mg/mL in tryptone , peptone, yeast extract , NH_4NO_3 to, urea respectively (Fig. 3). The xylanase activity of enzyme produced by the isolates in broth medium having tryptone, yeast extract, urea, ammonium nitrate and peptone as nitrogen sources showed that isolate NG-T163 produced maximum xylanase activity on yeast extract (1.5mg/mL). There was a gradual decrease from yeast extract to peptone in enzyme production having xylanase activity of 1.14mg/mL while NH_4NO_3 gave the lowest enzyme activity of 0.5mg/mL. In the case of isolate NG-T161, tryptone elicited the maximum enzyme activity of 1.16mg/mL which was followed by peptone with enzyme activity of 1.14mg/mL and yeast extract with xylanase activity of 1.0mg/mL. 0.8mg/mL enzyme activity was observed with NH_4NO_3 while the least enzyme activity of 0.6mg/mL was observed with urea. Earlier studies had reported that organic nitrogen sources have been found to be suitable for xylanase production in which yeast extract gave the best result (Battan et al. 2007). Similarly, the maximum production of xylanase in the presence of tryptone in this study for NG-T161 is in line with observation of Pang et al. (2006).

Effect of pH on xylanase production by the strains of *Trichoderma asperellum*

In Fig. 4, production of xylanase by these isolates was found to be dependent on pH and showed maximum enzyme production at slightly acidic range. Fungal isolate NG-T163 showed maximum xylanase activity of (3.8mg/mL) at a pH of 6.0 while the least xylanase activity occurred at a pH of 7.0 having xylanase activity of 1.04mg/m.L. In the case of NG-T161, the highest enzyme activity occurred at pH of 5.0 having enzyme activity of 3.04mg/ml while the least activity was observed at a pH of 7. The isolates have the same peak in enzyme production at a pH of 7. Sanghi, et al. (2010) and Pandey et al. (2014) had earlier reported that the optimum pH for xylanase activity was in the range of 5.0 to 6.0 further increase in pH decline the enzyme actitvity. The results in the work also agreed with observation.by Shah & Dutta (2005) that the highest xylanase titres for

fungal system occur generally at a pH of 5.0. Hoda et al. (2012), also reported that the maximum xylanase production by *Aspergillus niger* and *Trichoderma viride* are between pH 4.5-6.5.

Effect of incubation period on xylanase production in lignocellulosic wastes by the strains of *Trichoderma asperellum*

The ability of the two strains of fungi to produce xylanase in the presence of different lignocellulosic wastes indicates that each of the substrates elicited different susceptibility to the utilization by the fungi at various periods (Fig. 5). The agro-industrial wastes such as corn cob, sugarcane bagasse, groundnut shell and saw dust are good substrates for the induction of xylanolytic enzyme and the degree of xylanase produced by the isolates from each lignocellulosic waste might be attributed to the level of xylan present in each substrate (Hoda et al. 2012, Ahmad et al. 2012). Xylanase production started after 24h of incubation with all the substrates. Enzyme activity was highest after 96 h of incubation on isolate NG-T163 and NG-T161 having enzyme activity of 1.5 mg/mL and 1.56 mg/mL with saw dust. The lowest yield of enzyme was observed at 72h of incubation for both isolates. This is in contrast to the work done by Saleem et al. (2012) where it was reported that xylanase production reached its maximum peak at 48h of incubation while decline at 96h of incubation. The contrast might due to the differences in the type of substrates used. Corn cob is very reach in xylan and xylose. This may be responsible for the high yield of xylanase produced by corn cob. The highest enzyme activity (1.85 mg/mL) was produced at 72h of incubation for NG-T163 with corn cob while that of NGT161 was highest at 48h of incubation with xylanase activity of 2.48 mg/mL. The lowest yield of enzyme was produced at 24h and 48h of incubation for isolate NG-T163 while the lowest yield for isolate NG-T161 occurred at 24h and 72h of incubation. The observed variation might be connected with the different strains involved in the work. Desia & Iyer (2016) reported corn cob as a good substrate for high xylanase production by A. *niger* DX- 23.

Sugarcane bagasse contains high percentage of hemicellulose and this may be responsible for high enzyme yield. Enzyme activity was maximum at 24h for isolate NG-T163 having enzyme activity of 2.5 mg/mL with sugarcane bagasse while the highest enzyme yield occurred at 96 h of incubation for NG-T161. The lowest enzyme yield was produced at 72h for both isolate. These results have corroborated the studies of previous workers (Gautam et al. (2002), Irfan et al. (2014). Isolate NG-T163 while grown on groundnut shell produced the highest enzyme activity at 72 h of incubation while the highest value of xylanase (2.0 mg/mL) for isolate NG-T161 was recorded at 96 h of incubation. The lowest enzyme yield for isolate NG-T163 occurred at 24h while that of isolate NG-T161 occurred at 48h of incubation. The result obtained in the present work contrary to the work of Ashish et al. (2005) who reported groundnut shell as a poor substrate for the induction of cellulolytic enzyme by *Trichoderma viride*.

Incubation period has tremendous effect on the amount of enzyme produced by microorganisms. Although Fadel (2001), Hoda et al. (2012) reported that maximum xylanase was produced at 2 days of incubation of *Aspergillus niger* and *Trichoderma viride*, in this study, the maximum production of the enzyme varied with each of the strain of the fungi. The fluctuation of the enzyme activity recorded as the time of growth progressed might be as a result of utilization of xylose produced by the fungi as its accumulation lead to the inhibition of the enzyme production.

Time course degradation of lignocellulosic wastes with purified xylanase produced by the strains of *Trichoderma asperellum*

Degradative ability of purified xylanase from NG-T161 was consistently higher in all the wastes from 48h though 96h (Fig. 6). The highest enzyme activity was at 96h in all the substrates and for the two organisms. Although corncob has been reported to possess much xylan content among the three substrates used (corncob, banana peel and plantain peel) (Ruzene et al. 2008, Agama-Acevedo et al. 2016, Anil et al. 2016), maximum enzyme activity was observed with banana peel. This observation might be as a result of the source of the enzyme, being from the

organisms isolated from banana peel soil. It may also be due to the fact that xylan from different source exhibit significant variation in compositions and structure (Latif et al. 2006).

Conclusion

The need for removal of environmental pollution cursed by abundance of lignocellulosic wastes in Nigeria makes them sustainable in xylanase production for industrial use.

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