Original Research Article

Journal of Biological Research & Biotechnology

Bio-Research Vol. 19 No.1; pp.1192-1201 (2021). ISSN (print):1596-7409; eISSN (online):2705-3822

Partial Purification and Characterization of Endoxylanase from a fungus, *Leohumicola incrustata*

[1§]Adeoyo Olusegun Richard, [2]Pletschke Brett Ivan and [2]Dames Joanna Felicity

[1]Department of Microbiology, Adekune Ajasin University, P.M.B., 001, Akungba-Akoko, Ondo State, Nigeria.
[2]Department of Biochemistry and Microbiology, Rhodes University, P.O. Box 94, Grahamstown 6140, South Africa.

[§]Correspondence: Adeoyo Olusegun Richard. Email: olusegun.adeoyo@aaua.edu.ng

Abstract

Xylanases are glycoside hydrolases (GH) that degrade β-1,4-xylan, a linear polysaccharide found as hemicellulose in cell wall of plants. Endoxylanase (Endo-1,4-β-xylanase, EC 3.2.1.8) randomly catalyses xylan to produce varying short xylooligosaccharides (XOS). This study aimed to determine the characteristics of a partially purified endoxylanase from *Leohumicola incrustata*. Enzyme production was carried out using beechwood (BW) xylan, after which the cell-free crude filtrate was concentrated using the ammonium sulphate precipitation method. The hydrolysed products were analysed by thin-layer chromatography (TLC) and zymography. The result showed that the enzyme produced varying smaller-sized linear xylooligosaccharides with R_f values corresponding to those of xylobiose, xylotriose, xylotetraose, xylopentaose, xylohexaose and other higher oligomers. The endoxylanase had a molecular mass of 72 kDa. The enzyme is stable in the presence of K^+, Na^+, Ca^{2+}, Fe^{2+}, Mg^{2+}, Zn^{2+}, Co^{2+}, pH of 5.0 and temperature of 37^{o}C. However, the activity gradually decreased after 60 min at 50^{o}C and retained over 69% activity after 120 min, while at 60 and 70^{o}C, the enzyme activity sharply decreased (pre-incubation periods). Endoxylanase from *L. incrustata* is comparable to those of other microorganisms and should be considered an attractive candidate for future industrial applications.

Keywords: Endoxylanase, Enzyme catalytic properties, *Leohumicola incrustata,* Xylan.

https://dx.doi.org/10.4314/br.v19i1.2
Journal Homepage: ***http://www.bioresearch.com.ng.***
Publisher***: Faculty of Biological Sciences, University of Nigeria, Nsukka, Nigeria.***

INTRODUCTION

Xylan is an essential component of hemicellulosic polysaccharide in cell walls of most plants, making up to 7-12% and 15-30% of the total dry weight in softwood from gymnosperms and hardwood from angiosperms, respectively (Saha, 2003). Hardwood hemicellulose mainly consists of *O*-acetyl-L-4-*O*-methyl-glucuronic acid xylan, e.g., the content of hemicellulose in birchwood is approximately 35% (Chen, 2014; Sakthiselvan *et al.*, 2014). Xylan hemicellulose in softwoods is 4-*O*-methyl-glucuronic acid arabinose-xylan with almost no acetyl (Chen, 2014). Beechwood (BW) xylan from agricultural residues is an inexpensive and abundant raw material that could be used for oligosaccharide production. BW xylan consists of a backbone of *p*-1,4-linked D-xylopyranose residues, with side chains of 4-*O*-methyl-glucuronic acid attached to the C-2 position of xylose and *O*-acetyl groups at C-2 or C-3 positions (Freixo and De Pinho, 2002).

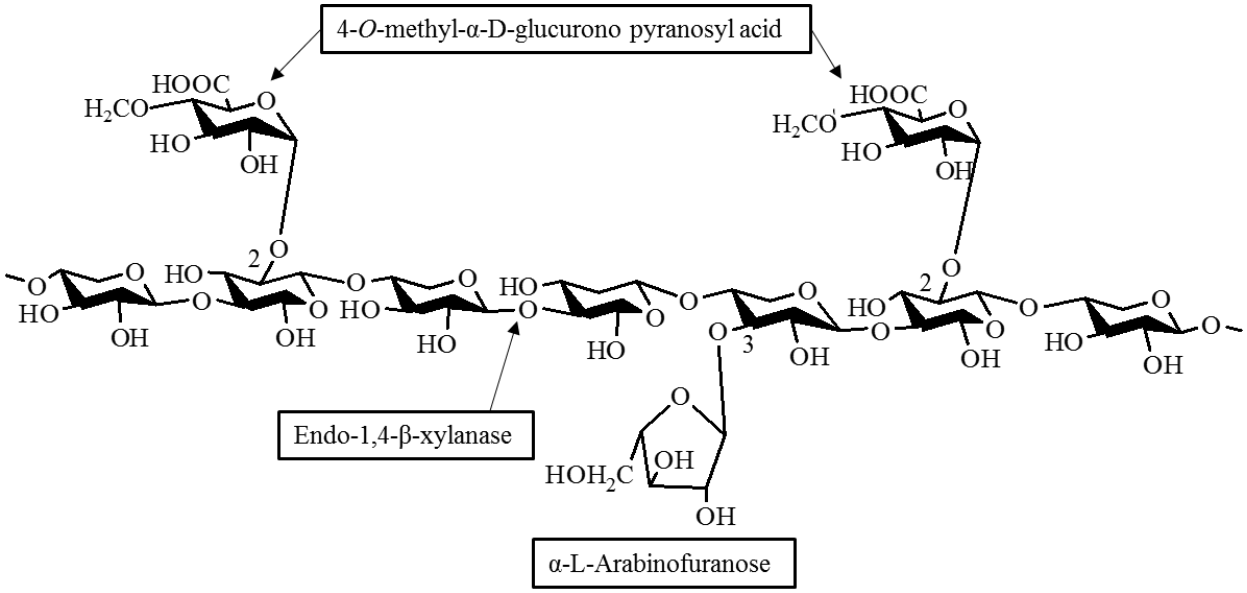

Figure 1: Action site of xylan-degrading endo-1,4-β-xylanase with numbers showing carbon atoms to which chemical groups are linked including acetyl chains (Adapted from Corral and Villaseñor-Ortega, 2006).

Xylanase is a class of enzyme that include endoxylanase (E.C 3.2.1.8), β-xylosidase (E.C 3.2.1.37), α-glucuronidase (E.C 3.2.1.139), α-arabinofuranosidase (E.C 3.2.1.55) and acetyl xylan esterase (E.C 3.1.1.72) (Juturu and Wu, 2011). Endoxylanase catalyses xylan to produce various short xylooligosaccharides (XOS), while β-xylosidase releases xylose residues from the non-reducing ends of XOS (Yun *et al.,* 2015). Endo-1,4-β-xylanase is the most important xylan degrading enzyme, it removes or cleaves the internal glycosidic linkages of the heteroxylan backbone, leading to a decreased degree of polymerisation of the substrate (Corral and Villaseñor-Ortega, 2006). Endo-1,4-β-xylanase cleaves the main chain only near a substituted region and acts only at uninterrupted sequences (Corral and Villaseñor-Ortega, 2006). Some novel xylanases degrade xylan at high temperatures and have contributed to the development of a highly efficient system for lignocellulose conversion (Goncalves *et al.,* 2015).

Xylanases are glycoside hydrolases (GH), and sequences based on GH classification has placed xylanase in two major families, 10 and 11. Other related GH families include 5, 8, 30 and 43 (Hong *et al.,* 2014; Chakdar *et al.,* 2016). Xylanases belonging to the GH10 family are composed mostly of endo-1,4-β-xylanases with a few endo-1,3-β-xylanases (biose, triose, and tetraose are the primary products, whereas the xylose, pentraose, and other oligosaccharides with more than five xylose units are produced in small quantities). They can hydrolyse cellulose and aryl β-D-cellobiosides. GH10 xylanases cleave the β-1,4-linkages that precede a β-1,3-linkage on both sides, but not the ones that immediately follow β-1,3-linkages. GH11 members are monospecific, as they consist exclusively of an appropriate enzyme that cleaves internal β-1,4-xylosidic linkages only.

Their catalytic ability is lower than those of enzymes in the GH10 class. The action of the GH11 family enzymes is solely on D-xylose containing substrates, and they cannot cleave cellulose or aryl β-D-cellobiosides (Chakdar *et al.,* 2016). The GH11 family cleaves un-substituted regions of the backbone because they cannot attack the xylosidic linkage towards the nonreducing end (next to a branched xylose). Most bacterial xylanases belong to the GH10 family while fungal xylanases majorly belong to the GH11 family (Liu *et al.,* 2012).

Interest in carbohydrate-active enzymes has increased over the years because of their potential application. Xylanases are used in food, feed, bleaching (pulp and paper) industries, and could also be utilised in improving the effectiveness of detergent in cleaning, biochemical and biofuel production (Juturu and Wu, 2011; Yun *et al.,* 2015). This study aimed to determine the characteristics of a partially purified endoxylanase from *L. incrustata*.

MATERIALS AND METHODS

Culture

Leohumicola incrustata (Isolate code ChemRU330 / Genbank Accession Number MF374380 / The South African National Collection of Fungi Accession Number PPRI 17268) was obtained from Mycorrhizal Research Laboratory, Rhodes University, Grahamstown. The isolate was preserved on PDA at a temperature of 4^{o}C throughout the study period.

Enzyme production

Beechwood (BW) xylan (1%, w/v, Lot # 141202, Megazyme, Bray, Ireland) was weighed and added to a salt solution composed of: (g L^{-1}): malt extract 3.0; $(NH_4)_2HPO_4$ 0.25; $MgSO_4.7H_2O$ 0.15; $CaCl_2$ 0.05; NaCl 0.025; $ZnSO_4.7H_2O$ 0.003; thiamine-HCl 100 µg L^{-1} and 1.2 mL of $FeCl_3$ (1%, w/v). Production medium of 100 mL was distributed into each of 150 mL Erlenmeyer flasks; the contents were thoroughly mixed and sterilized at 121^{o}C for 15 min. The sterilized medium was inoculated with two discs of 5 mm mycelial plugs of the fungus. A non-inoculated medium was used as a control. Growth was allowed to proceed at 28^{o}C in the dark for three weeks in a rotary incubator shaker at 150 rpm. After incubation, cultures were homogenized using IKA's ULTRA-TURRAX homogenizer (20,000 rpm), and centrifugation was performed at 10,000 x *g* for 15 min to get crude enzyme filtrates (supernatant) using Beckman Coulter Avanti-J high-speed centrifuge. The pellet containing the mycelia was re-suspended in 10 mL water and filtered using vacuum suction filtering system to recoup the mycelia on a filter paper (Adeoyo *et al.,* 2018).

Ammonium sulphate precipitation and dialysis of crude enzyme extract

Cell-free crude filtrate was concentrated and optimised using the ammonium sulphate precipitation method (Kamble and Jadhav, 2012). The precipitation was carried out by diluting 120 mL crude enzyme extract into $(NH_4)_2SO_4$ with a concentration of 80% (w/v). The pellet and filtrate were separated by centrifugation at 6000 x *g* for 15 min at 4^{o}C. The precipitated enzyme in the pellet was diluted with 10 mL acetate buffer (pH 5.0). Dialysis of the partially purified enzyme was performed using a pre-treated dialysis bag (10 kDa cut-off). The partially purified enzyme (10 mL) was dialysed against 0.1 M acetate buffer (pH 5.0) at 4^{o}C with three changes of buffer according to the method described by Kusuda *et al.* (2004).

TLC analysis of hydrolytic products

The hydrolysed products of xylan were analysed by the thin-layer chromatography (TLC) using silica gel plates 60G F254 HPTLC (Merck, Darmstadt Germany). BW xylan was used as the substrate; the reaction mixture was incubated at 50^{o}C for 24 h. An aliquot (80 µl) sample was collected after boiling and centrifugation at 6000 x *g* for 5 min. A 5 µl of each aliquot with xylo-oligomer standard, substrate, and enzyme blanks were spotted onto a TLC plate. The plate was subsequently developed with n-butanol: acetic acid: water (2:1:1, v/v/v) (Yun *et al.,* 2015). Plate was developed twice for 3 h 15 min, then for 2 h 15 min and was briefly submerged in a staining solution of methanol containing 5% phosphoric acid, and 0.3% α-naphthol. To visualise the xylo-oligomers, the plate was heated for 10 min at 120°C in an oven. The xylooligosaccharide mixture consisted of xylose (Sigma-Aldrich, Lot # SLBK7809V), xylobiose (Megazyme, Lot # 130502), xylotriose (Megazyme, Lot # 140803), xylotetraose (Megazyme, Lot # 150604), xylopentaose (Megazyme, Lot # 150605), and xylohexaose (Megazyme, Lot # 151206).

Zymography

Zymogram analysis was performed using a modified zymographic method (Ratanakhanokchai *et al.,* 1999). The culture

supernatant in the sample application buffer was boiled for 2 min at 95°C and was followed by electrophoresis on a 10% sodium dodecyl sulphate - polyacrylamide gel electrophoresis (SDS-PAGE) gel containing 1% BW xylan. After electrophoresis, the gel was soaked in 2.5% (v/v) Triton X-100 with gentle shaking which removed the SDS and renatured the proteins in the gel for 45 min at 4°C. The gel was then washed with 0.01 M acetate buffer (pH 5.0) and incubated for 1 h at 37°C. The gel was soaked in 0.1% Congo red solution for 30 min at room temperature and washed with 1 M NaCl until the excess dye was removed from the active band. A Bio-Rad ChemiDoc X-Ray Spectrometer (XRS) system was used to capture photographic images.

Enzyme assay

A dinitrosalicylic acid (DNS) assay (Miller, 1959) was conducted by adding 1% (w/v) BW xylan to a volume of 10 mL sodium acetate buffer (pH 5.0) in a Schott bottle and boiled for 30 s. A volume of 100 µL of crude enzyme and uninoculated control was added to 300 µL of BW xylan-containing medium in triplicate, while the blank contained 400 µL buffer. All samples were incubated at 37°C for 1 h, followed by centrifugation at 6000×*g* for 2 min. A 300 µL aliquot of DNS was added to 150 µL of each supernatant sample. This was followed by boiling on a heating block at 100°C for 5 min after which it was cooled on ice for 5 min. A volume of 250 µL of each sample was placed into each well of a 96-well plate and read with the aid of a spectrophotometer (BioTek's Synergy Mx) at a wavelength of 540 nm. The supernatant was taken to determine the reducing sugar using DNS assay with xylose as a standard. Enzyme activity was measured using 1% BW xylan in acetate buffer (pH 5.0) for 1 h at 37°C. The reducing sugars released were assayed using the DNS method of Miller (1959).

Protein estimation

Protein content was estimated according to the method described by Bradford (1976) using bovine serum albumin (BSA, Lot # A9647, Sigma-Aldrich) as a standard.

Effect pH of endoxylanase activity and stability

The optimum pH was obtained by assaying the partially purified enzyme in buffer at different pH (1.0-9.0) prepared in 0.1 M buffer having pH values of 1.0, 2.0 (hydrochloric acid-potassium chloride); 3.0, 4.0, and 5.0 (citrate-phosphate); 6.0, 7.0 (phosphate); 8.0 and 9.0 (Tris-HCl). For enzyme stability, the enzyme was preincubated in acetate buffer (pH 5.0) at 37°C. The optimum pH was determined by assaying enzyme activity at 37°C for 1 h using the DNS method (Miller, 1959).

Effect of temperature on endoxylanase activity and stability

The optimum temperature was obtained by incubating the enzyme under different temperatures (4, 20, 30, 40, 50, 60, 70, and 80°C, while stability was tested by pre-incubating the enzyme at 37, 50, 60, and 70°C. The enzyme activity was determined every 6 h for 30 h.

Effect of metal ions and chemicals on the activity of the endoxylanase

Metal ions (K^+, Na^+, Ca^{2+}, Fe^{2+}, Mg^{2+}, Zn^{2+}, Co^{2+}, Cu^{2+} Al^{3+}, Hg^{2+}, Cd^{2+}, and Mn^{2+} (all supplied in chloride form) were used to determine enzyme stability. Each metal ion was used at a concentration of either 1, 5, or 10 mM, while incubation was performed at 50°C for 1 h. The following compounds, sodium azide (NaN_3), ammonium chloride (NH_4Cl), ethylenediaminetetraacetic acid (EDTA), SDS, dimethyl sulfoxide (DMSO), and indole-3-acetic acid (IAA) were also tested for their inhibitory effect on enzyme activity.

Substrate specificity

A 10 mg/ml each of either BW xylan, CMC, starch, glycogen, microcrystalline cellulose (Avicel) or chitin was used to determine the substrate specificity of the xylanase. Each substrate was incubated with the xylanase extract at 50°C for 1 h (pH 5.0). Activity was determined as previously described.

Statistical analysis

All experiments were conducted in triplicate and analysed using one-way ANOVA. Error bars were represented as the standard errors of the means (±SEM).

RESULTS

Partial purification of endoxylanase

Table 2 shows partial purification table, where the protein was precipitated with 80% ammonium sulphate, dialysed against acetate buffer and concentrated using an Amicon ultrafiltration unit. The enzyme was partially purified to 49.6 fold with

a specific activity of 1.57 U/mg protein and a recovery yield of 77%. Figure 2 shows the SDS-PAGE and zymogram of the crude extract from *Leohumicola incrustata*. The partially purified sample gave a single band of yellow against the red colour of the Congo red used for gel staining. The molecular weight of the endoxylanase was estimated by plotting a relative migration distance (R_f) graph based on the electrophoretic mobilities of endoxylanase and of the reference standard (R_f) on SDS-PAGE with the corresponding zymographic position. The partially purified endoxylanase was observed to have a molecular weight of 72 kDa.

Table 2: Purification table of endo-1,4-β-xylanase from *Leohumicola incrustata*

Step	Protein (mg/ml)	Total protein (mg)	Total activity (U)	Specific activity (U/mg)	Purification fold	Yield (%)
Cell-free filtrate	245.93	29511.11	936.90	0.03	1.0	100
Ammonium sulphate/Dialysis	41.50	460.68	725.35	1.57	49.6	77

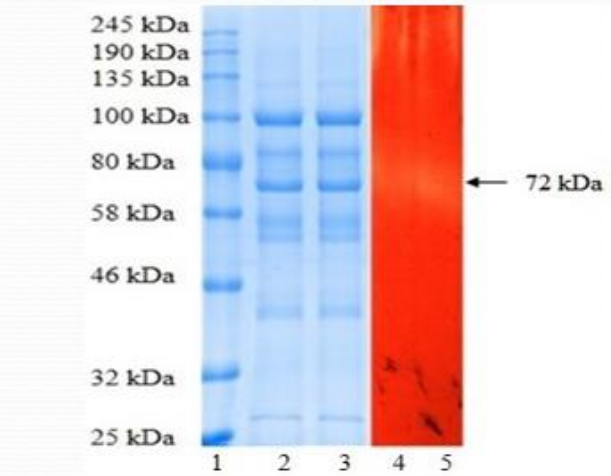

Figure 2: The SDS-PAGE and endo-1,4-β-xylanase zymogram of a *Leohumicola incrustata* partially purified enzyme. Lane 1 contains a colour pre-stained standard, broad range (BioLabs), lanes 2-3 contain partially purified enzyme and lanes 4-5 contain β-1,4-endoxylanase zymogram (Congo red staining).

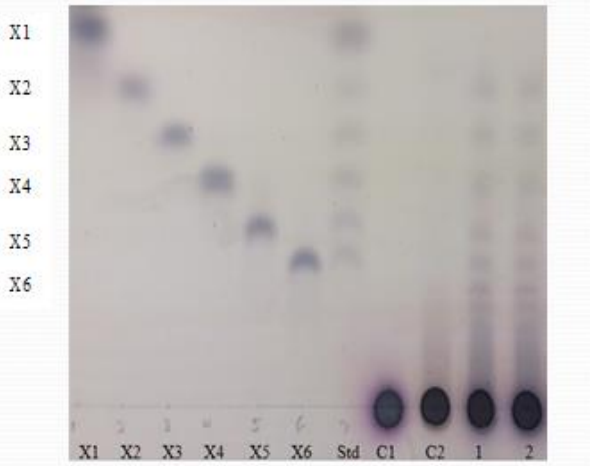

Figure 3: Thin-layer chromatograms of hydrolyzates using endo-1,4-β-xylanase from *Leohumicola incrustata*. Standards (Std); xylose (X1), xylobiose (X2), xylotriose (X3), xylotetraose (X4), xylopentaose (X5), and xylohexaose (X6), substrate control (C1), enzyme control (C2), sample (1-2).

Thin layer chromatographic analysis of the xylanase

Beechwood xylan (1%) was incubated with the enzyme for 24 h to assure maximum hydrolysis. The mixtures were analysed by thin layer chromatography (TLC), and the hydrolytic products were compared to those of the standards. The result showed that the tested enzyme liberated varying smaller-sized linear xylooligosaccharides with R_f values corresponding to those of xylobiose, xylotriose, xylotetraose, xylopentaose, xylohexaose and other higher oligomers (Figure 3).

The effect of pH on endo-1,4-β-xylanase activity and stability

The pH of any medium plays a crucial role in influencing enzyme production and activity. Figure 4 shows that pH 5.0 had the highest activity of 1.11 U/mg protein. Low enzyme activity was found at a pH of 2.0 and 8.0 while the activity at the pH of 1.0 was significantly low. The optimum pH for the endoxylanase activity from *L. incrustata* was 5.0, and the activity gradually decreased below this pH. Also, it was observed that the enzyme activity was stable at pH of 5.0 for 6 h (Figure 5), after which the activity started to decrease gradually in a similar faction to the activity at the other pH. After 24 h of pre-incubation, the enzyme retained over 70% of its activity at a pH of 5.0 at 37°C.

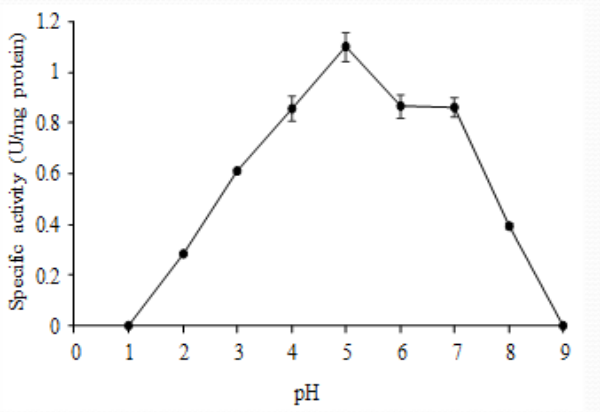

Figure 4: Effect of pH on endo-1,4-β-xylanase activity. The enzyme was incubated at 37°C for 1 h. All error bars are represented as the standard errors of the means (SEM).

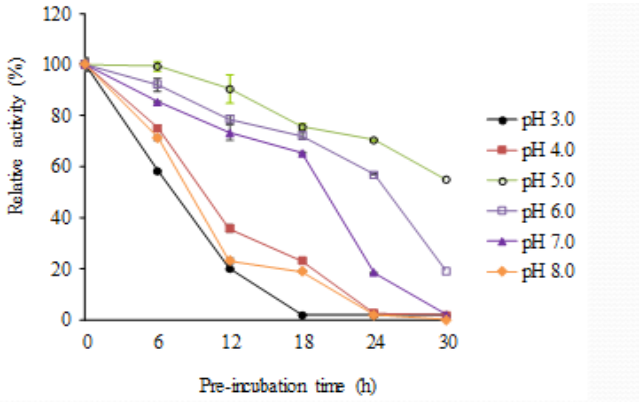

Figure 5: Effect of pH on the stability of endo-1,4-β-xylanase. The enzyme was incubated at 37°C for 1 h. All error bars are represented as the standard errors of the means (SEM).

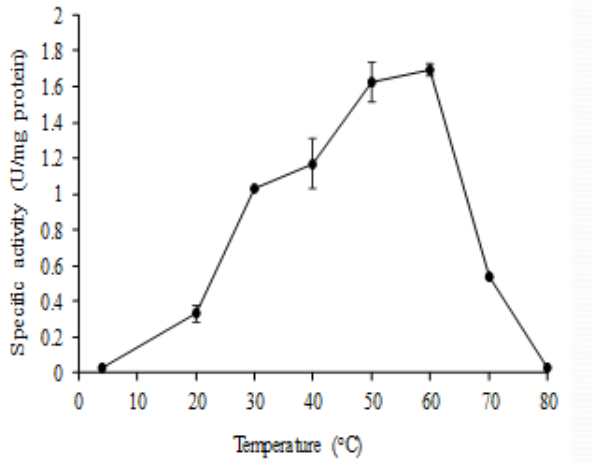

Figure 6: Effect of temperature on the endo-1,4-β-xylanase activity. The enzyme was incubated at 4 to 80°C for 1 h (pH 5.0). All error bars are represented as the standard errors of the means (SEM).

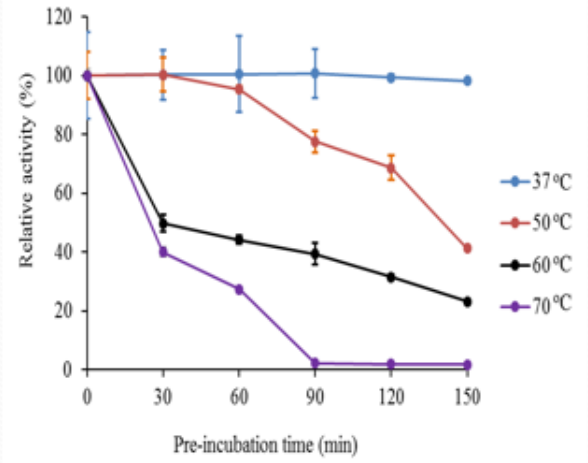

Figure 7:Thermal stability of the endo-1,4-β-xylanase activity. The enzyme was pre-incubated for 30, 60, 90, 120, and 150 min before the assay was carried out at 50°C for 1 h (pH 5.0). All error bars are represented as the standard errors of the means (SEM).

Effect of temperature on endo-1,4-β-xylanase activity

Figure 6 shows that the optimal temperature was obtained between 60°C (1.69 U/mg protein). The xylanase was stable at 37°C. However, the activity of endoxylanase gradually decreased after 1 h at 50°C and retained over 69% activity after 120 min, while at 60 and 70°C, the enzyme activity sharply decreased (pre-incubation periods) (Figure 7).

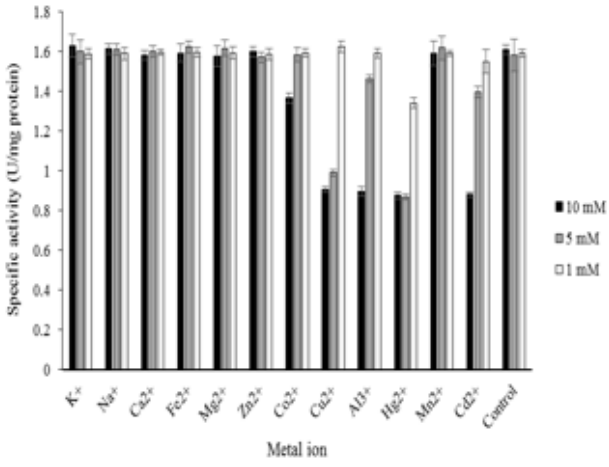

Figure 8: Effect of metals on the endo-1,4-β-xylanase activity. The enzyme was incubated in either 1, 5, or 10 mM of K^+, Na^+, Ca^{2+}, Fe^{2+}, Mg^{2+}, Zn^{2+}, Co^{2+} Cu^{2+} Al^{3+}, Hg^{2+}, Cd^{2+}, and Mn^{2+} at 50°C for 1 h (pH 5.0). All error bars are represented as the standard errors of the means (SEM).

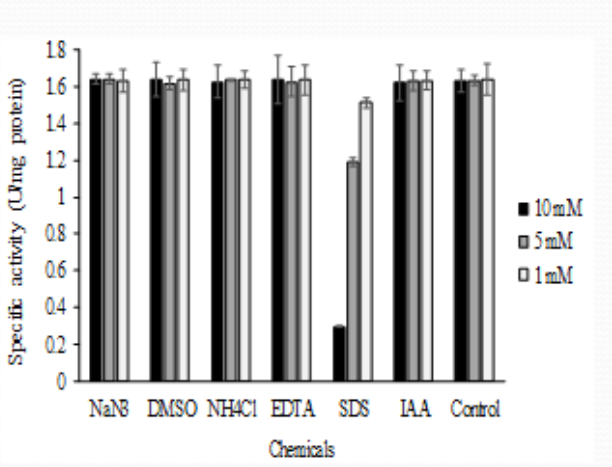

Figure 9: Effect of chemicals on the endo-1,4-β-xylanase activity. The enzyme was incubated in either 1, 5, or 10 mM of NaN_3, DMSO, NH_4Cl, EDTA, SDS and IAA at 50°C for 1 h (pH 5.0). All error bars are represented as the standard errors of the means (SEM).

Effect of metal ions and chemicals on the activity of the endo-1,4-β-xylanase

Metal ions such as Cu^{2+}, Al^{3+}, Hg^{2+} and Cd^{2+} inhibited enzyme activity significantly at a concentration of either 1, 5 or 10 mM, while Cobalt had an effect only at a concentration of 10 mM (Figure 8). Also, for most chemicals used in this experiment (Figure 9), only SDS showed an inhibitory effect on endoglucanase activity at 1, 5 and 10 mM concentrations.

Substrate specificity

The substrate (1%) of either BW xylan, CMC, starch, glycogen, Avicel or chitin was used to determine specificity. Figure 10 shows that endo-1,4-β-xylanase was specific for two substrates - BW xylan and CMC with values of 1.61 and 0.42 U/mg protein, respectively.

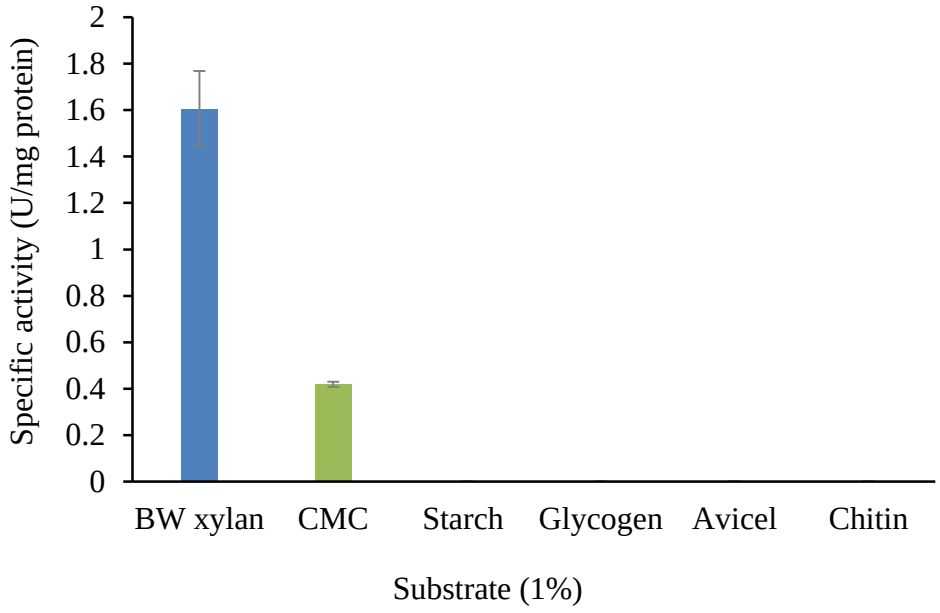

Figure 10. Substrate specificity of endo-1,4-β-xylanase activity. All error bars are represented as the standard errors of the means (SEM).

DISCUSSION

The study revealed a single protein band with a molecular weight (MW) of 72 kDa (Figure 2). This corroborates a report on an endo-1,4-β-xylanase from some fungi that displayed a MW ranging from 8.5 to 85 kDa (Polizeli *et al.,* 2005). The capacity of endo-1,4-β-xylanase to degrade the substrates (BW xylan) was demonstrated by analysing the hydrolysates through a TLC method. The qualitative identification showed different XOS (xylobiose, xylotriose, xylotetraose, xylopentaose, xylohexaose and other higher oligomers as products of hydrolysis) while no XOS was produced where the enzyme was not included. The hydrolytic profile observed in the *L. incrustata* was similar to that of *Aspergillus niger* (Takahashi *et al.,* 2013) and *Penicillium oxalicum* (Liao *et al.,* 2015).

Xylanases obtained from fungal sources are known to be active and stable in the acidic range of pH (Burke and Cairney, 1997a). The pH study indicated that pH 5.0 was the optimum while optimum stability was observed at a pH between 5.0 and 7.0. Also at this pH, the enzyme retained 70% of its activity after incubation for 24 h at 37^{o}C. This is in contrast to a report that an endo-1,4-β-xylanase from the ericoid mycorrhizal fungus (*Hymenoscyphus ericae*) had an optimum pH of 4.5 and was stable between pH 3.5-4.0 (Burke and Cairney, 1997a). According to Burke and Cairney (1997a), one of the environmental factors regulating enzyme activity at the mycorrhizal-host root interface is pH, which tends to reduce enzyme activity and growth when outside the optimum or permissible range. Also, endoxylanase of *L. incrustata* had optimal activity at a temperature of 60^{o}C (pH 5.0), was more stable at 50^{o}C, retaining about 69% activity after 2 h. These results confirmed that the endoxylanase from *L. incrustata* compared well with those of commonly used *Trichoderma* (Abbas *et al.,* 2012) and *Aspergillus* species (Subramaniyan and Prema, 2002).

The effects of metals, detergents, and other chemicals were evaluated, and the partially purified enzyme showed strong stability in the presence of most metals used (K^{+}, Na^{+}, Ca^{2+}, Fe^{2+}, Mg^{2+}, Zn^{2+}, Co^{2+} and Mn^{2+}) except for Cu^{2+} Al^{3+}, Hg^{2+} and Cd^{2+} (Figure 8). The study was at variance with a report that endoxylanase was inhibited in the presence of Co^{2+} and Mn^{2+} (Chen *et al.,* 2006; Hmida-Sayari *et al.,* 2012). Also, substrate specificity is a key indicator to determine the efficiency and products of hydrolysis because not all of the xylosidic linkages in the heteroxylans are readily accessible to a particular xylanase (Gírio *et al.,* 2010). The degradation profile of BW xylan by xylanase monitored by the TLC analysis revealed that the hydrolytic products were longer xylooligomers. This result revealed that the xylanase produced from *L. incrustata* had similar catalytic properties to those in the GH11 family, a characteristic feature of fungal xylanases (Liu *et al.,* 2012). The efficient utilisation of xylanases relies on a proper understanding of their substrate specificity and the complex structures of heteroxylans. A good number of studies on the three-dimensional structures of xylanases from different GH families in complex with the substrate provide insight into the various mechanisms through which xylanases bind and hydrolyse structurally different heteroxylans and xylooligosaccharides (Pollet *et al.,* 2010).

CONCLUSION

In conclusion, endoxylanase from *L. incrustata* compared well with those reported for those of other fungal endoxylanase. Further purification of this enzyme to homogeneity would show far more accurate results.
Finally, the unique properties exhibited by xylanase from *L. incrustata* had placed the fungus among the attractive candidates for future industrial applications. For example, it can be used to produce xylanase for the conversion of agricultural residues into other useful bio-based products such as ethanol.

Acknowledgements

The authors are grateful to Dr. Christine Bizabani, Rhodes University, Mycorrhizal Research Laboratory, Grahamstown, South Africa for the kind gift of the isolates used in this study.

Conflict of interests

Authors have no conflict of interest to declare.

Funding

This study was funded by Rhodes University, a Sandisa Imbewu Grant, South Africa, and Adekunle Ajasin University/Tertiary Education Trust Fund (TETFund), Nigeria.

REFERENCES

Abbas, A., Ahmad, S., Mushtaq, Z. and Jamil, A. (2012). Partial purification and characterisation of xylanase from *Trichoderma harzianum*. *Journal of the Chemical Society of Pakistan,* **34**:1455-1459.

Bradford, M.M. (1976). A rapid and sensitive method for the quantitation microgram quantities of protein utilising the principle of protein-dye binding. *Analytical Biochemistry,* **72**: 248–254.

Burke, R.M., Cairney and J.W.G. (1997a). Purification and characterisation of a β-l,4-endoxylanase from the ericoid mycorrhizal fungus *Hymenoscyphus ericae*. *New Phytologist,* **135**:345–352.

Chakdar, H., Kumar, M., Pandiyan, K., Singh, A., Nanjappan, K., Kashyap, P.L. and Srivastava, A.K. (2016). Bacterial xylanases: biology to biotechnology. *3 Biotechnology,* **6**: 1-15.

Chen, H. (2014). Biotechnology of lignocellulose: Theory and practice, Biotechnology of Lignocellulose: Theory and Practice. pp. 25-71.

Chen, H.G., Yan, X., Liu, X.Y., Wang, M.D., Huang, H.M., Jia, X.C. and Wang, J.A. (2006). Purification and characterisation of novel bifunctional xylanase, XynIII, isolated from *Aspergillus niger* A-25. *Journal of Microbiology Biotechnology,* **16**: 1132-1138.

Corral, L.O. and Villaseñor-Ortega, F. (2006). Xylanases. *Advances in Agric-Food Biotechnology*, **661**: 305-322.

Freixo, M.R. and De Pinho, M.N. (2002). Enzymatic hydrolysis of beechwood xylan in a membrane reactor. *Desalination* **149**: 237-242.

Gírio, F.M., Fonseca, C., Carvalheiro, F., Duarte, L.C., Marques, S. and Bogel-Łukasik, R. (2010). Hemicelluloses for fuel ethanol: A review. *Bioresource Technology*, **101**: 4775–4800.

Goncalves, G.A.L., Takasugi, Y., Jia, L., Mori, Y., Noda, S., Tanaka, T., Ichinose, H. and Kamiya, N. (2015). Synergistic effect and application of xylanases as accessory enzymes to enhance the hydrolysis of pretreated bagasse. *Enzyme Microbial Technology*,**72**: 16-24.

Hmida-Sayari, A., Taktek, S., Elgharbi, F. and Bejar, S. (2012). Biochemical characterisation, cloning and molecular modelling of a detergent and organic solvent-stable family 11 xylanases from the newly isolated *Aspergillus niger* US368 strain. *Process Biochemistry* **47**: 1839-1847.

Hong, P.Y., Iakiviak, M., Dodd, D., Zhang, M., Mackie, R.I. and Cann, I. (2014). Two new xylanases with different substrate specificities from the human gut bacterium *Bacteroides intestinalis* DSM 17393. *Applied and Environmental Microbiology,* **80**:2084-2093.

Juturu, V. and Wu, J.C. (2011). Microbial xylanases: Engineering, production and industrial applications.*Biotechnology Advances,* **30**: 1219-1227.

Kamble, R.D. and Jadhav, A.R. (2012). Isolation, purification, and characterisation of xylanase produced by a new species of *Bacillus* in solid state fermentation. *International Journal of Microbiology,* **2012**: 1-8.

Kusuda, M., Ueda, M., Konishi, Y., Matsuzawa, K., Shirasaka, N., Nakazawa, M., Miyatake, K. and Terashita, T. (2004). Characterisation of extracellular glucoamylase from the ectomycorrhizal mushroom *Lyophyllum shimeji*. *Mycoscience*, **45**:383-389.

Liao, H., Zheng, H., Li, S., Wei, Z., Mei, X., Ma, H., Shen, Q. and Xu, Y. (2015). Functional diversity and properties of multiple xylanases from *Penicillium oxalicum* GZ-2. *Scientific Reports* **5**: 12631.

Liu, L., Wang, L., Zhang, Z., Guo, X., Li, X. and Chen, H. (2012). Domain-swapping of mesophilic xylanase with hyper-thermophilic glucanase. *BMC Biotechnology*, **12**: 1-7.

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

Polizeli, M.L.T.M., Rizzatti, A.C.S., Monti, R., Terenzi, H.F., Jorge, J.A. and Amorim, D.S. (2005). Xylanases from fungi: properties and industrial applications. *Applied Microbiology and Biotechnology,* **67**: 577-591.

Ratanakhanokchai, K., Kyu, K.L. and Tanticharoen, M. (1999). Purification and properties of a xylan-binding endoxylanase from alkaliphilic *Bacillus* sp. strain K-1. *Applied and Environmental Microbiology,* **65**: 694-697.

Saha, B.C. (2003). Hemicellulose bioconversion. *Journal of Industrial Microbiology and Biotechnology,* **30**: 279-291.

Sakthiselvan, P., Naveena, B. and Partha, N. (2014). Molecular characterisation of a xylanase-producing fungus isolated from fouled soil. *Brazilian Journal of Microbiology,* **45**: 1293-1302.

Subramaniyan, S. and Prema, P. (2002). Biotechnology of microbial xylanases: enzymology, molecular biology and application. *Critical Reviews in Biotechnology,* **22**: 33-46.

Takahashi, Y., Kawabata, H. and Murakami, S. (2013). Analysis of functional xylanases in xylan degradation by *Aspergillus niger* E-1 and characterisation of the GH family 10 xylanase XynVII. *SpringerPlus*, **2**: 447.

Yun, K., Yopi and Meryandini, A. 2015. Characterisation of xylanase activity produced by *Paenibacillus* sp. XJ18 from TNBD Jambi, Indonesia. *Hayati Journal of Biosciences,* **22**: 20-26.