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Research article

Production and biochemical characterization of partially purified cellulase-free, thermo-acidophilic endoxylanase from *Lysinibacillus fusiformis* strain TB7 using kolanut husk as feedstock

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ABSTRACT

Xylanases have become very important enzymes in many industrial processes for the valorization of xylan-rich lignocellulosic wastes. Here, some physicochemical and kinetic properties of a purified endoxylanase produced on kolanut husk-based medium by *Lysinibacillus fusiformis* are presented. The crude enzyme solution was first subjected to precipitation with solid ammonium sulphate and further purified on DEAE-Sephadex A-50 anion-exchange and Sephadex G-100 gel filtration columns chromatography prior to biochemical characterization. The purified endoxylanase was 21 kDa as determined by sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) and was thermostable, exhibiting optimum activity at 60 °C and pH 5.0. The K_m and V_{max} were respectively estimated to be 29.5 mg/ml and 125 μmol/min/ml using Birchwood xylan as substrate. Activity of the enzyme was enhanced by Na^+, Ca^{2+}, Mn^{2+}, Mg^{2+} and K^+ at concentration of 5 mM but inhibited by Hg^{2+}, Cu^{2+}, Pb^{2+}, Fe^{3+}, EDTA, SDS and Urea. The purified endoxylanase showed high hydrolytic activity on Birchwood xylan and kolanut husk but extremely poor or no activity on carboxymethyl cellulose, starch or pectin. This *L. fusiformis* strain TB7 endoxylanase has desirable properties useful for biotechnological applications in laundry, fuels, feeds, paper and pulp industries.

1. Introduction

The earth is endowed with many natural resources which humans continue to explore for beneficial purposes. For instance, lignocellulosic biomasses, the earth's most abundant repository of carbon, represent the most abundant renewable organic resource in soil (Sánchez, 2009) and have been identified as cheap resources for the production of "second generation" biofuels without competing with human food chain (Sharma et al., 2016). Meanwhile, lignocellulosic biomass is a complex matrix comprised of cellulose (35–50%), hemicellulose (20–35%), lignin (15–20%), and 15–20% other minor components such as ash, protein, minerals and pectin (Pauly and Keegstra, 2010; Mood et al., 2013).

Hemicelluloses are a group of plant-derived heterogeneous polymers of various sugars found in the cell walls in association with cellulose and lignin. They are a mixture of highly branched, low molecular weight homo- and heteropolymers comprised of anhydro-β-(1–4)-D-xylopyranose, glucopyranose, mannopyranose, and galactopyranose units (Gurunathan et al., 2015). Xylan is the most important natural hemicellulose (Kumar et al., 2018) that requires the action of several enzymes grouped as xylanolytic enzymes for complete degradation.

Endo-1,4-β-xylanases (1,4-β-D-xylan xylohydrolase; EC 3.2.1.8) are the most important xylan-degrading enzymes that catalyze the hydrolysis of the 1,4-β-D-xylosidic linkages in xylans, yielding various xylooligosaccharides and small molecules such as monosaccharides, disaccharide and trisaccharides of β-D-xylopyranosyl (Bajpai, 2014). Xylanases are used in a multitude of biotechnological applications including seed germination and fruit ripening, degumming, coffee processing, brewing and fruit juice clarification.

Despite the vast application, the market share for this enzyme is low due to intolerance to industrial processing conditions and high

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Table 1. Summary of purification of endo-1,4-β-xylanase from *L. fusiformis* TB7.

Purification step	Total activity (U)	Total protein (mg)	Specific activity (U/mg)	Yield (%)	Purification fold
Crude	333182.12	2003.77	166.28	100.00	1.00
Ammonium sulphate precipitation (60%)	42059.63	165.73	253.78	12.62	1.53
DEAE-Sephadex A-50 (ion-exchange)	25903.29	63.32	409.12	7.77	2.46
Sephadex G-100 (gel filtration)	25611.35	39.18	653.72	7.69	3.93

production cost. Selection of suitable, low-cost substrate is a key approach to reducing the production cost of industrially important enzymes, amidst many. Quite a number of researches have been devoted to finding cheap and alternative substrates such as agro-waste biomasses, forest residues and industrial wastes as feedstock for xylanolytic enzyme production.

The kolanut husk is an agricultural waste with rich lignocellulosic and nutritional properties (Adeyi, 2010). Kolanut seed extract has been employed in different processes: flavour for soft drinks, production of heat-tolerant chocolate bars, used as snack in traditional ceremonies and its essential oils are used in pharmaceutical industries for drug production (Asogwa et al., 2006; Jayeola et al., 2018) while the by-products (agro-residue) are discarded indiscriminately into the environment. Utilization of kolanut residues as substrate for the production of enzymes such as endoxylanase will enhance its clean-up from the environment in much the same way agricultural wastes such as wheat bran, corn-cob, rice husk have been used as good, cheap and alternative substrates for the production of xylanases at different fermentation conditions (Mardawati et al., 2020).

However, structural variation and complexity of polysaccharides embedded in lignin matrix, as seen in an ideal (raw) lignocellulosic biomass, is a major constraint to achieving high productivity of enzyme in microbial fermentation. Microorganisms have developed various mechanisms to deconstruct lignocellulosic materials by utilizing complex proteins. Commercially, lignocellulose-degrading enzymes have been produced by both fungi and bacteria with special preference for bacteria because they possess sophisticated glycoside hydrolases which establish synergy with organismal mixture of extreme niches and have a faster

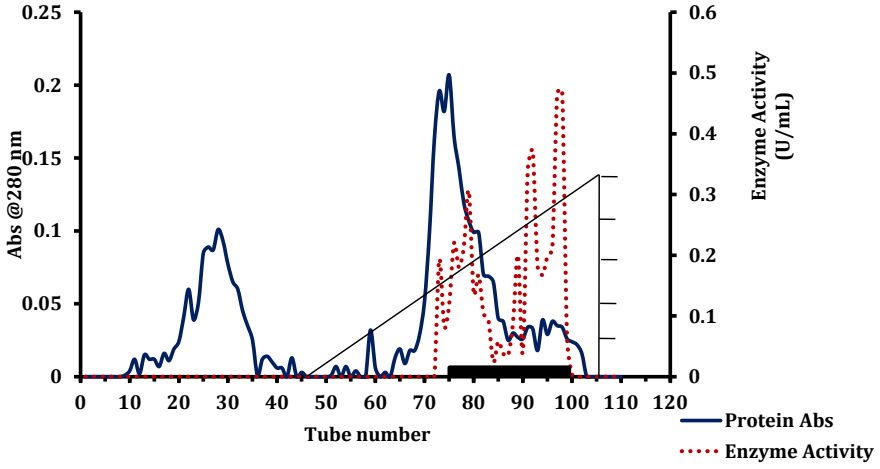

Figure 1. Elution profile of endoxylanase from *L. fusiformis* TB7 on ion-exchange column chromatography. ▬ Indicates the pooled sample.

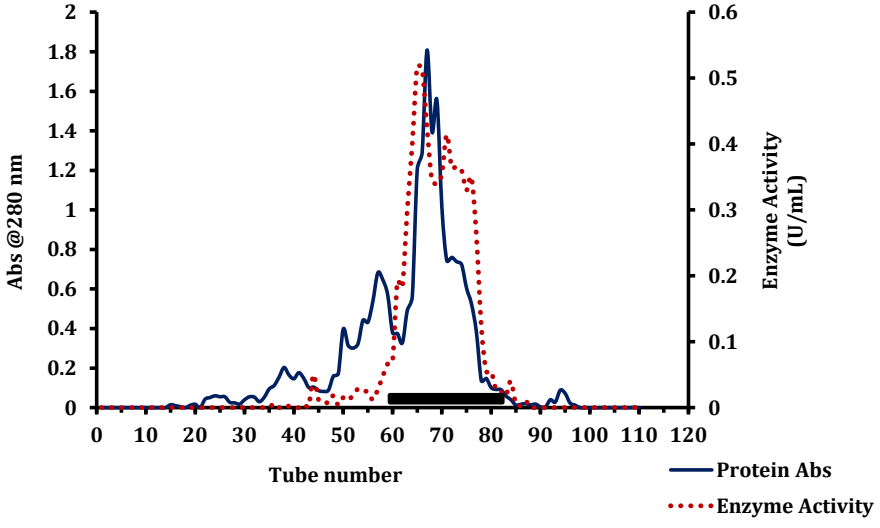

Figure 2. Elution profile of endoxylanase from *L. fusiformis* TB7 on gel filtration column chromatography.

growth rate than fungi (Raze et al., 2019). Therefore, this research aims at the optimal production, purification and biochemical characterization (kinetic and physicochemical) of bacterial endoxylanase using *Lysinibacillus fusiformis* TB7, mass cultured on a medium containing kolanut husk as low-cost carbon source.

2. Materials and methods

2.1. Sample collection and preparation

The kolanut husk, agro-waste samples were obtained from a kolanut plantation in Ile-Ife, Osun State, Nigeria and authenticated at the Department of Crop, Soil and Pest Management of the Federal University of Technology, Akure, Nigeria. The kolanut husks were rinsed with distilled water, cut into smaller pieces, washed with hot water before being microwaved at 300 °C for 5 min. The husks were grinded into a relatively homogenous powdery form and well stored prior to use.

2.2. Isolation and identification of microorganisms

The bacterium used in this study was isolated from the soil environment of a kolanut plantation and identified through an extensive biochemical test as well as the molecular gene sequence analysis of its 16S rRNA to ascertain its identity. This microbial species coded strain TB7 in the Laboratory was confirmed to be *Lysinibacillus fusiformis* and deposited in NCBI database with GenBank accession number **MH085463.1** (Fabunmi et al., 2018). The organism was maintained on nutrient agar and sub-cultured periodically all through this study.

2.3. Endoxylanase production by submerged fermentation

The production of endoxylanase was carried out under submerged fermentation at optimized culture condition. The mineral salt media (1 l) was prepared to contain (g/l) 1.0, xylan; 0.1, K_2HPO_4; 0.5, $CaCl_2$; 0.5, $MgSO_4.7H_2O$; 2.0, peptone; 2.5, yeast extract; 5.0, sucrose; 2.5, KNO_3; $FeSO_4.7H_2O$ and 15 g pulverised kolanut husk in a set of shake-flasks. The media was sterilized at 121 °C for 15 min before inoculating with 24 h old *L. fusiformis* strain TB7 broth. The shake-flasks were thereafter incubated under constant shaking condition at 150 rpm and 35 °C for 24 h. The crude culture was subjected to centrifugation at 10,000 rpm using a refrigerated centrifuge set at 4 °C for 15 min to remove the cells and unspent media components. The supernatant obtained from the centrifugation process was taken as extracellular crude enzyme for further analysis.

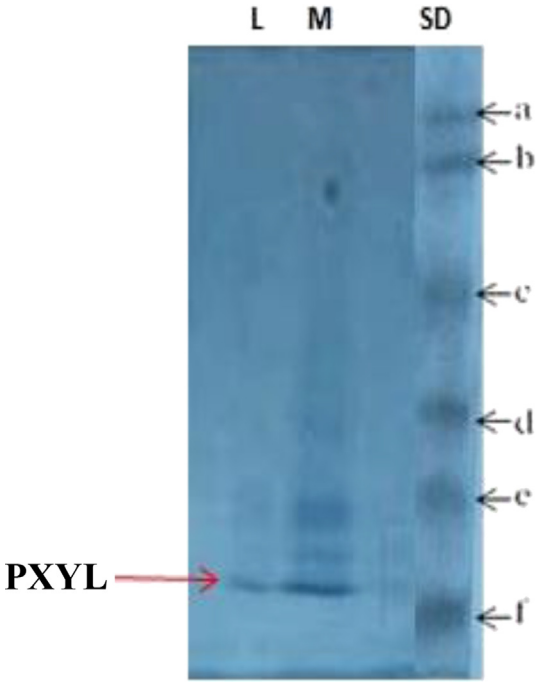

Figure 3. SDS-PAGE analysis of purified endoxylanase from *L. fusiformis* TB7. Lane SD: Standard protein marker; a = Phosphorylase b (113,142 Da), b = Bovine serum Albumin (81,353 Da), c = Ovalbumin (47,045 Da), d = Carbonic Anhydrase (34,173 Da), e = Trypsin Inhibitor (27,259 Da), f = Lysozyme (17,671 Da); Lane L: Purified Xylanase; Lane M: Crude Xylanase.

2.4. Assay of endoxylanase activity

An assay method earlier described by Bhalla et al. was used for the determination of xylanase activity with birchwood xylan as substrate. Five hundred microliter of 1% (w/v) birchwood xylan prepared in 100 mM phosphate buffer (pH 6.8) was added to a tube containing 500 μL of enzyme solution and the reaction mixture was incubated at 40 °C for 30 min. The enzyme-substrate reaction was terminated and the amount of reducing sugar released was determined using DNS method. A unit of endoxylanase activity was defined as the amount of enzyme that could liberate 1 μmol (μmol) of xylose as reducing sugar under the specified assay conditions.

2.5. Determination of protein concentration

The concentration of protein was determined by the method of Bradford (1976) using Bovine Serum Albumin (BSA) as the standard protein.

2.6. Enzyme purification

The supernatant of the crude enzyme solution was subjected to protein precipitation by adding salt of ammonium sulphate with gentle stirring on ice until it attained 60% saturation and allowed to stay overnight. The precipitated protein was obtained by centrifugation at 10,000 rpm for 10 min and dialyzed extensively against phosphate buffer at 4 °C using Por™ 3 RC dialysis membrane tube (3500 da molecular weight cut-off). The dialysate was loaded on an anion-exchange column containing DEAE-Sephadex A-50 resin (2.5 cm × 40 cm) previously equilibrated with phosphate buffer (50 mM, pH 6.8). Proteins were eluted from the column with the same buffer at flow rate of 60 mL/h while bound proteins were obtained using gradient elution with 1 M NaCl solution. The presence of protein in the eluted fractions was

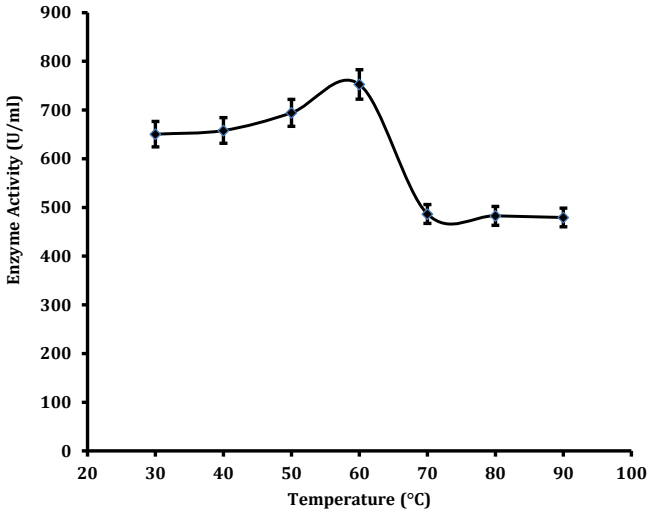

Figure 4. Effect of temperature on the activity of purified endoxylanase from *L. fusiformis* TB7.

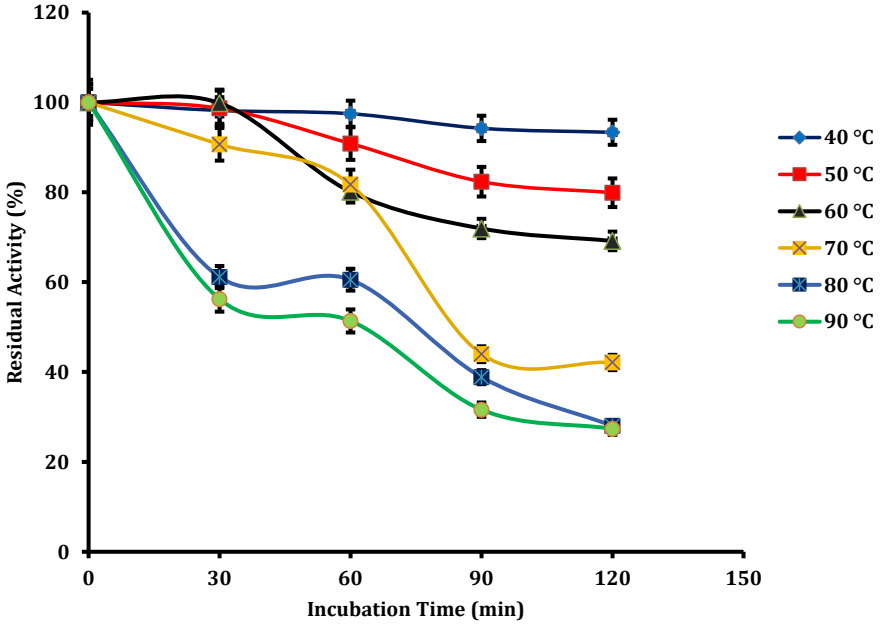

Figure 5. Effect of temperature on the stability of endoxylanase purified from *L. fusiformis* TB7.

monitored by measuring the absorbance at 280 nm (Shimadzu, UV 1800) and endoxylanase activity determined as earlier described. Active tubes were pooled, concentrated using an ultrafiltration system before loading on a Sephadex G-100 gel filtration column (2.5 × 75 cm, flow rate of 30 mL/h) already equilibrated with the same buffer. Protein elution was monitored by measuring absorbance of collected fractions at 280 nm and assayed for the activity of endoxylanase. The active tubes were pooled and used as purified enzyme for biochemical studies.

2.7. Determination of molecular weight

The subunit molecular weight of the partially purified xylanase were determined by sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) according to the method of Laemmli (1970) using the Tris-glycine-SDS buffer system. The gel was stained with Coomassie brilliant blue R-250 and the visible protein bands of the enzyme and protein marker were observed after de-staining process.

2.8. Physicochemical characterization of purified endoxylanase

2.8.1. The effect of pH on enzyme activity and stability

The activity of endoxylanase from *Lysinibacillus fusiformis* strain TB7 as a function of solution pH was studied by carrying out the assay procedure earlier described by incubating 500 μL of enzyme solution and 500 μL substrate (1% birchwood xylan) prepared in different buffer solutions (pH 3–10). Sodium citrate buffer was used for pH 3.0–4.0; sodium acetate (4.5–5.5); sodium phosphate (pH 6.0–7.0); Tris–HCl buffer (pH 8.0–10). Enzyme stability was studied by pre-incubating the enzyme in appropriate buffer solution at ratio 1:1 for a period of 180 min. Aliquot of enzyme from the enzyme-buffer mixture was withdrawn every 30 min to measure the residual enzyme activity following the assay procedure earlier described.

2.8.2. The effect of temperature on enzyme activity and stability

The activity of the purified *L. fusiformis* strain TB7 endoxylanase was determined by incubating 500 μL of enzyme solution and 500 μL substrate (1% birchwood xylan) prepared in phosphate buffer solution (100 mM, pH 6.8) at varying reaction temperature (30–90 °C) for 30 min. For stability studies, the purified enzyme was pre-incubated at respective temperature (40–90 °C) for 120 min with aliquot of enzyme withdrawn (500 μL) every 30 min from the pre-incubated enzyme solution at each temperature and the residual activity measured accordingly following the assay procedure earlier described.

2.8.3. The effect of cations on enzyme activity

The activity of *L. fusiformis* strain TB7 endoxylanase was determined in the presence of some metal ions using their chloride salts (KCl, $CuCl_2$, $CaCl_2$, $PbCl_2$, $AlCl_3$, $FeCl_3$, NaCl, $MnCl_2$, $HgCl_2$ and $MgCl_2$) at 5 mM salt concentration relative to absence of the metal ions. The enzyme-substrate reaction was carried out by incubating 500 μL of the substrate (1% w/v birchwood xylan) prepared in phosphate buffer (100 mM, pH 6.8) with 500 μL of enzyme solution respective solutions (with/without the metal ion), for 30 min at 45 °C and the enzyme activity was estimated as earlier described.

2.8.4. The effect of some chemical agents on enzyme activity

The activity of the purified *L. fusiformis* strain TB7 endoxylanase was determined following the earlier described assay procedure in the presence of some chemical agents such as ethylenediamine tetraacetic acid (EDTA), sodium dodecyl sulphate (SDS) urea and β-mercaptoethanol at 5

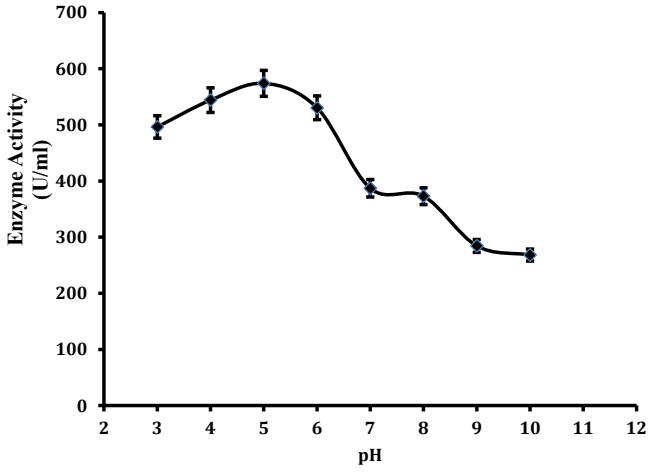

Figure 6. Effect of pH on the activity of purified endoxylanase from *L. fusiformis* TB7.

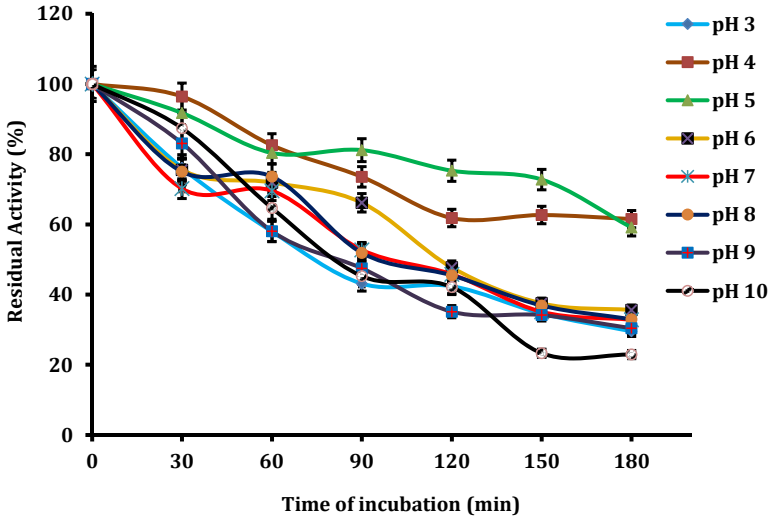

Figure 7. Effect of pH on the stability of purified endoxylanase from *L. fusiformis* TB7.

mM concentrations. The activities relative to a reaction without any agent were recorded after 30 min of incubation at 45 °C.

2.9. Kinetic studies

2.9.1. Determination of kinetic parameters

Initial reaction rate of the enzyme-substrate reaction was measured for this *L. fusiformis* strain TB7 endoxylanase with different concentrations of Birchwood xylan (1–10 mg/mL) as the substrate. The enzyme-substrate reaction was carried out under same reaction conditions of 40 °C for 30 min as earlier described. The kinetic parameters (K_m and V_{max}) of the purified xylanase were estimated from the double reciprocal plot of Lineweaver and Burk (1934).

2.9.2. Substrate specificity of the purified xylanase

The activity of the purified *L. fusiformis* strain TB7 endoxylanase was determined on different substrates including Birchwood xylan, carboxyl methyl cellulose (CMC), starch and pectin. Each substrate was prepared in phosphate buffer (50 mM, pH 6.8) and the assay procedure used was same as previously described.

Table 2. Effect of metal ions on the activity of endo-1,4-β-xylanase from *L. fusiformis* TB7.

Metal ion/chemical agent (5 mM)	Relative activity (%)
Control	100 ± 0.0
K^+	112.88 ± 1.3
Cu^{2+}	17.17 ± 0.7
Ca^{2+}	120.64 ± 0.9
Pb^{2+}	14.61 ± 0.5
Mg^{2+}	118.91 ± 1.5
Al^{3+}	73.34 ± 1.3
Fe^{3+}	16.07 ± 1.0
Na^+	106.72 ± 2.3
Mn^{2+}	105.33 ± 2.1
Hg^{2+}	5.96 ± 0.5
EDTA	46.46 ± 0.8
SDS	54.57 ± 0.6
Urea	89.19 ± 1.3
β-Mercaptoethanol	107.72 ± 2.1

2.10. Statistical analysis

The experiments were mostly performed in triplicates and the significant difference of mean values was assessed with one-way analysis of variance (ANOVA) with test of significance $p > 0.05$. The values were expressed as mean ± standard deviation. All graphs including kinetic analysis graphs were drawn using either Microsoft Excel spreadsheet or GraphPad Prism v8.0 from GraphPad Software (San Diego, CA).

3. Results and discussion

3.1. Endoxylanase production and purification

Xylanolytic enzymes bring about the hydrolysis of complex xylan biomolecule, into simple monomers and their usefulness in industrial processes is gaining much attention. *L. fusiformis* strain TB7 exhibited excellent production of xylanase under submerged fermentation using kolanut pod husk, an agro-waste, as substrate. As summarised in Table 1, about 4-fold purification with 653.7 U/mg specific activity and approximately 8% recovery were obtained after a 3-step purification process comprising precipitation with ammonium sulphate, ion-exchange (Figure 1) and gel filtration chromatography (Figure 2). Different strategies including combination of two or three methods have been used in the purification of xylanases from *Bacillus* sp. in recent times (Mittal et al., 2013; Geetha and Gunasekaran, 2017) and other microorganisms (Sanjivkumar et al., 2017; Yadav et al., 2018).

3.2. SDS-PAGE analysis

A molecular weight (MW) of 21 kDa obtained for this *L. fusiformis* strain TB7 endoxylanase from SDS-PAGE analysis suggests that the protein is monomeric (Figure 3) and similar to reports of xylanases from other bacterial strains including *B. pumilus* SV-85S (Nagar et al., 2012a), *Bacillus* sp. SV-34S (Mittal et al., 2013) and *Paenibacillus macquariensis* RC 1819 (Sharma et al., 2013). Low molecular weight xylanases within the range of 21 kDa as obtained in this study are particularly important in paper and pulp industries owing to the fact that smaller enzymes penetrate the fibre wall structure and works more efficiently in altering the pulp properties. Some researchers have reported similar xylanases with molecular weight within the range of 15.7–26.4 kDa (Rahayu et al., 2008; Chi et al., 2010; Shrinivas et al., 2010). However, xylanases having

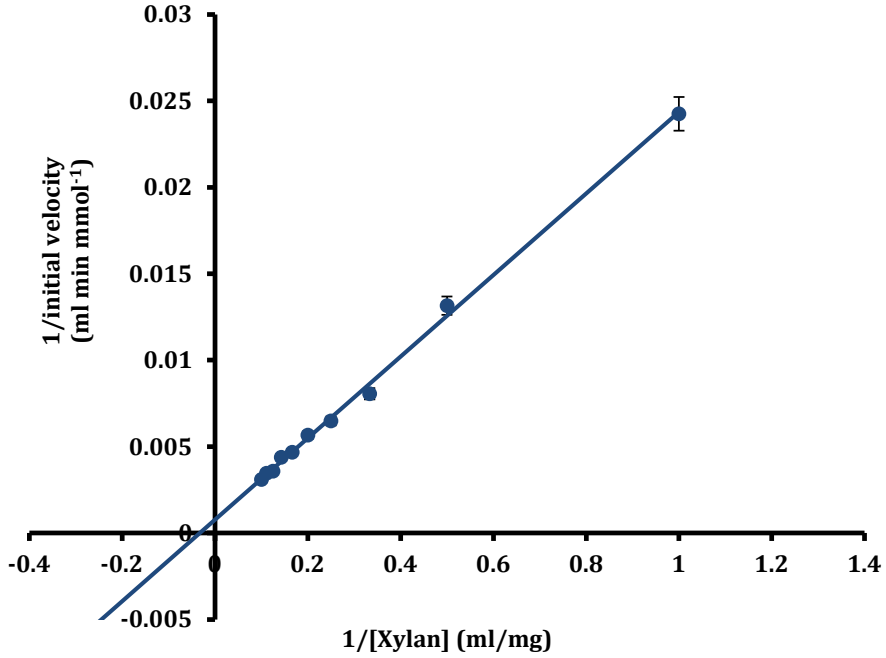

Figure 8. Double reciprocal plot of endoxylanase activity on birchwood xylan substrate.

higher molecular weights (40–100 kDa) has also been reported (Verma et al., 2013).

3.3. The effect of temperature on the xylanase activity and stability

This purified *L. fusiformis* strain TB7 endoxylanase displayed high activity of over 650 U/mL at 30–50 °C and showed optimum activity of 752 U/mL at 60 °C. It however exhibited reduction in activity at 70–90 °C with over remaining 400 U/mL (Figure 4). An optimum temperature of 40 °C was reported for endoxylanase activity from *Bacillus* (Rahayu et al., 2008). Interestingly, this purified *L. fusiformis* strain TB7 endoxylanase exhibited maximum thermal stability by retaining almost all its initial activity 94% at 40 °C while it retained over 70 and 80% activity after 120 min of pre-incubation at 50 and 60 °C, respectively. However, it showed sharp decline in its thermal stability at 70 °C with about 40% remaining residual activity and 80 and 90 °C leaving residual activity of less than 30% after 2 h incubation (Figure 5). Xylanase produced from *Paenibacillus* sp. HPL-002 was only thermally stable for 60 min at 40 °C but sharply decreased after 10 min incubation at 50 °C (Park et al., 2012). Generally, xylanases from bacteria are more thermally stable than those from fungi (Kulkarni et al., 1999) and have been of interest to industrialists to minimize cost accrued from excess enzyme acquisition due to easy loss in enzyme activity. Temperature strongly affects chemical reaction by affecting the structural integrity of the enzyme molecule, subsequently affecting enzyme activity (Meryandini et al., 2006).

3.4. The effect of pH on xylanase activity and stability

Activity of about 496 U/mL was recorded at the acidic pH 3.0 which increased to 536 U/mL at pH 4. Maximum activity was obtained at pH 5.0 while further increase in the pH to the alkaline region led to a decline in enzyme activity (Figure 6). The optimum pH 5.0 for activity of this *L. fusiformis* strain TB7 xylanase is at variant to the alkaline pH reported for many xylanases; pH 8.6 for *Paenibacillus macquariensis* RC 1819 (Sharma et al., 2013) while *Geobacillus thermodenitrificans* TSAAI (Verma et al., 2013), *B. halodurans* TSEV-1 and *Paenibacillus* sp. HPL-002 (Park et al., 2012) have optima activity at pH 9. Meanwhile, xylanase optimum activity at acidic pH is an excellent attribute for industrial applications such as feed and foods. This purified *L. fusiformis* strain TB7 xylanase was very stable in acidic environment and notably, a residual activity of 60% was obtained at acidic pH 4.0–5.0 after a 3-hour pre-incubation period. This *L. fusiformis* strain TB7 xylanase also was stable at near neutral pH 7, retaining about 65% of initial activity after 1 h incubation in this pH. The enzyme was relatively stable in the alkaline region (8.0–10.0), as it maintained about half of its initial activity after 90 min of incubation (Figure 7). Xylanases from *Bacillus* species are known to have wide-range pH stability as obtained in this study, corroborating reports of Bocchini et al. (2002) for *B. circulans* D1 xylanase (pH 5.5–10.5). The xylanase from *Bacillus subtilis* ASH was stable at pH 6.0–9.0 (Sanghi et al., 2010) while the xylanase from *B. pumilus* SV-85S was reported to be stable at pH 5–11 (Nagar et al., 2012b).

3.5. Effect of metallic ions and chemical agents on the activity of xylanase

The activity of the purified endoxylanase from *L. fusiformis* strain TB7 was stimulated by K^+, Ca^{2+}, Mg^{2+}, Na^{2+} and Mn^{2+} with percentage increase of activity relative to the control (Table 2). Adhyaru et al. (2017) and Kumar et al. (2015) have both reported that the xylanase from *Bacillus altitudedinus* was activated by Ca^{2+}, Mn^{2+}, K^+ and Na^{2+}; whereas the activity of the enzyme was strongly inhibited in the presence of Al^{3+}, Hg^{2+}, Pb^{2+}, Fe^{3+} and Cu^{2+}. Interestingly, Fe^{2+} and Cu^{2+} ions have been reported to activate xylanase from *Geobacillus* sp. Strain DUSELR13 (Bibra et al., 2018) and *Streptomyces olivaceus* (MSU3) (Sanjivkumar et al., 2017) while Hg^{2+} was reported by Nagar et al. (2012b) and Mittal et al. (2013) to inhibit xylanase.

EDTA and SDS at 5 mM concentration caused reduction in the activity of the purified endoxylanase with 46.46% and 54.56% remaining relative activity respectively while the enzyme was slightly inhibited by urea

Table 3. Substrate specificity of *L. fusiformis* TB7 endo-1,4-β-xylanase.

Substrate	% Relative activity
Birchwood xylan	100.00 ± 0.01
Crude xylan from Kolanut husk	76.89 ± 1.21
Carboxymethyl cellulose (CMC)	0.5 ± 0.02
Soluble Starch	0.00
Pectin	0.72 ± 0.03

but was moderately activated by β-mercaptoethanol (Table 2). The positive influence of addictive such as β-mercaptoethanol has been reported by Fialho et al. (2004). Inhibition of the enzyme could be attributed to reduction in the disulphide bonds essential for maintaining the enzyme conformation.

3.6. Kinetic parameters

As calculated from the Lineweaver-Burk plot shown in Figure 8, the values of the K_m and V_{max} of the purified endoxylanase from *Lysinibacillus fusiformis* strain TB7 were 29.50 mg/ml and 125 U/min/mg respectively when birchwood xylan was used as substrate. Mittal et al. (2013) reported K_m and V_{max} values of 3.7 mg/ml and 133.33 IU/mL respectively for the xylanase purified from *Bacillus* sp. SV-34S while a K_m value of 5.6 mg/ml and V_{max} of 433 μL/min/mg were reported by Kumar et al. (2017) for *Bacillus amyloliquefaciens*. The Km is a measure of how tightly the enzyme binds its substrate. The purified *L. fusiformis* strain TB7 endoxylanase characterized in this study seem to bind the birchwood xylan used as substrate less tightly and probably releases it quickly during reactions owing to the fact that the velocity of the reaction is high, reaching a maximum at 125 U/min/mg protein. This is a desirable property in industrial enzymes to facilitate quick product development at minimal time frame.

3.7. Substrate specificity

The partially purified endoxylanase showed high activity towards birchwood xylan as a model substrate followed by crude Kolanut husk as an ideal substrate. The purified enzyme was not active on carboxymethyl cellulose (CMC) indicating that the partially purified enzyme is free of cellulase activity. The partially purified endoxylanase did not exhibit any hydrolytic activity towards the α-1, 4-glycosidic bonds present in both starch and pectin (Table 3).

4. Conclusion

The utilization of kolanut pod husk as low-cost biomass substrate gave a high yield of endo-1,4-β-xylanase from *Lysinibacillus fusiformis* strain TB7. This purified *L. fusiformis* strain TB7 endoxylanase possesses enviable properties that make it potentially useful in many industries for the hydrolysis of xylan. The enzyme was thermostable and had broad pH range stability, with optimum activity at pH 5.0 and temperature of 60 °C. Its typically low molecular mass has good applications in biobleaching and deinking processes in paper and pulp industries.

Declarations

Author contribution statement

Temitope B. Fabunmi; Adeyemi O. Ayodeji: Contributed reagents, materials, analysis tools or data.

Oladipo O. Olaniyi: Analyzed and interpreted the data.

Daniel Juwon Arotupin: Conceived and designed the experiments.

Omisore, Suliat O.: Performed the experiments; Wrote the paper.

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Data availability statement

The data that has been used is confidential.

Declaration of interest's statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

References

Adhyaru, D.N., Bhatt, N.S., Modi, H.A., Divecha, J., 2017. Cellulase-free thermo-alkalisolvent- stable xylanase from *Bacillus altitudinis* DHN8: over-production through statistical approach, purification and bio-deinking/bio-bleaching potential. Biocatal. Agric. Biotechnol. 12, 220–227.

Adeyi, O., 2010. Proximate composition of some agricultural wastes in Nigeria and their potential use in activated carbon production. J. Appl. Sci. Environ. Manag. 14, 55–58.

Asogwa, E.U., Anikwe, J.C., Mokwunye, F.C., 2006. Kola production and utilization for economic development. Afric. Scientist 7, 217–222.

Bajpai, P., 2014. Sources, production and classification of xylanases. In: Xylanolytic Enzyme. Academic press, Elsevier, Tokyo, pp. 43–52.

Bibra, M., Kunreddy, V., Sani, R., 2018. Thermostable xylanase production by *Geobacillus* sp. strain DUSELR13 and its application in ethanol production with lignocellulosic biomass. Microorganisms 6, 93.

Bocchini, D.A., Alves-Prado, H.F., Baida, L.C., Roberto, I.C., Gomes, E., Da Silva, R., 2002. Optimization of xylanase production by *Bacillus circulans* D1 in submerged fermentation using response surface methodology. Process Biochem. 38, 727–731.

Chi, W.J., Park, D.Y., Chang, Y.K., Hong, S.K., 2010. A novel alkaliphilic xylanase from the newly isolated mesophilic *Bacillus* sp. MX47: production, purification and characterization. Appl. Biochem. Biotechnol. 168, 899–909.

Fabunmi, T.B., Arotupin, D.J., Adegunloye, D.V., 2018. Study of bacterial associated with kolanut soil plantation and waste looking at their benefits to man and his environment. Nig. J. of Tech. 37, 1128–1136.

Geetha, K., Gunasekaran, P., 2017. Purification of endoxylanase from *Bacillus pumilus* B20 for production of prebiotic xylooligosaccharide syrup; an in vitro study. Iran. J. Biotechnol. 15, 232–240.

Gurunathan, T., Mohanty, S., Naya, S.K., 2015. A review of the recent developments in biocomposites based on natural fibres and their application perspectives. Compos. Appl. Sci. Manuf. 77, 1–25.

Jayeola, C.O., Adebowale, B.A., Yahaya, L.E., Ogunwolu, S.O., Olubamiwa, O., 2018. Production of bioactive compounds from waste. In: Therapeutic, Probiotic, and Unconventional Foods. Elsevier Inc, pp. 317–340.

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

Kumar, S., Haq, I., Prakash, J., Singh, S.K., Mishra, S., Raj, A., 2017. Purification, characterization and thermostability improvement of xylanase from Bacillus amyloliquefaciens and its application in pre-bleaching of kraft pulp. 3 Biotech 7, 20.

Kumar, P.S., Yaashikaa, P.R., Saravanan, A., 2018. Isolation, characterization and purification of xylanase producing bacteria from sea sediment. Biocatal. Agric. Biotechnol. 13, 299–303.

Laemmli, U.K., 1970. Cleavage of structural proteins during assembly of the head of bacteriophage T4. Nature 227, 680–685.

Mardawati, E., Sinurat, Y., Yuliana, T., 2020. Production of crude xylanase from *Trichoderma* sp. using *Reutealis trisperma* exocarp substrate in solid state fermentation. IOP Conf. Ser. Earth Environ. Sci. 515 (1–9), 012024.

Meryandini, A., Hendarwin, T., Saprudin, D., Lestari, Y., 2006. Characterization of xylanase *Streptomyces* spp. SKK1-8. HAYATI. J. Biosci. 13, 151–155.

Mittal, A., Nagar, S., Gupta, V.K., 2013. Production and purification of high levels of cellulase-free bacterial xylanase by Bacillus sp. SV-34S using agro-residue. Ann. Microbiol. 63, 1157–1167.

Mood, S.H., Golfeshan, A.H., Tabatabaei, M., Salehi Jouzani, G., Nafaji, G.H., Gholami, M., Ardjmand, M., 2013. Lignocellulosic biomass to bioethanol, a comprehensive review with a focus on pretreatment. Renew. Sustain. Energy Rev. 27, 77–93.

Nagar, S., Mittal, A., Gupta, V.K., 2012b. Enzymatic clarification of fruit juices (apple, pineapple and tomato) using purified *Bacillus pumilus* SV-85S xylanase. Biotechnol. Bioproc. Eng. 17, 1165–1175.

Nagar, S., Jain, R.K., Thakur, V.V., Gupta, V.K., 2012a. Biobleaching application of cellulase poor and alkali stable xylanase from *Bacillus pumilus* SV-85S. 5 Biotech.

Park, N.J., Lim, H.K., Song, H.Y., Kim, D.R., Lee, K.I., Hwang, I.T., 2012. Cloning, expression and characterization of an alkalophillic Endo-1,4-β-xylanase from *Paenibacillus* sp. HPL-002. Bioresources 7 (1), 727.

Pauly, M., Keegstra, K., 2010. Plant cell wall polymers as precursors for biofuels. Curr. Opin. Plant Biol. 13 (3), 305–312.

Rahayu, P., Setyahadi, S., Harmita, D., 2008. Production and purification of xylanase from Indonesian isolate *Bacillus* sp. AQ-1 grown on bunch palm oil. J. Microbiol. Indonesia. 3, 23–26.

Raze, A., Bashir, S., Tabassum, R., 2019. Evaluation of cellulases and xylanases production from *Bacillus spp*. isolated from buffalo digestive system. Kafkas Univ. Vet. Fak. Derg. 25 (1), 39–46.

Sánchez, C., 2009. Lignocellulosic residues: biodegradation and bioconversion by fungi. Biotechnol. Adv. 27, 185–194.

Sanghi, A., Garg, N., Gupta, V.K., Kuhad, R.C., 2010. One-step purification and characterization of cellulase-free xylanase produced by alkalophilic *Bacillus subtilis* ASH. Braz. J. Microbiol. 41, 467–476.

Sanjivkumar, M., Silambarasan, T., Palavesam, A., Immanuel, G., 2017. Biosynthesis, purification and characterization of β-1,4-xylanase from a novel mangrove associated actinobacterium *Streptomyces olivaceus* (MSU3) and its applications. Protein Expr. Purif. 130, 1–12.

Sharma, N., Ranchan, S., Pathania, S., 2013. Production, purification and characterization of cellulase free-xylanase by *Bacillus coagulans* B30 using lignocellulosic forest wastes with different pretreatment methods. J. Agro Aliment. Proc. Technol. 19, 28–36.

Sharma, V., Sharma, S., Kuila, A., 2016. A review on current technological advancement of lignocellulosic bioethanol production. J. Appl. Biotechnol. Bioeng. 1 (2), 1–7.

Shrinivas, D., Savitha, G., Raviranjan, K., Naik, G.R., 2010. A highly thermostable alkaline cellulase-free xylanase from thermoalkalophilic *Bacillus* sp. JB 99 suitable for paper and pulp industry: purification and characterization. Appl. Biochem. Biotechnol. 162, 2049–2057.

Verma, D., Anand, A., Satyanaryana, T., 2013. Thermostable and alkali-stable endoxylanase of the extremely thermophilic bacterium *Geobacillus thermodenitrificans* TSAA1: cloning, expression, characteristics and its applicability in generating xylooligosaccharides and fermentable sugars. Appl. Biochem. Biotechnol. 170, 119–130.

Yadav, P., Maharjan, J., Korpole, S., et al., 2018. Production, purification, and characterization of thermostable alkaline xylanase from *Anoxybacillus kamchatkensis* NASTPD13. Front. Bioeng. Biotechnol. 6, 65.