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Research article

Production and characterization of acidophilic xylanase from wood degrading white rot fungus by solid-state fermentation of wheat straw

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ABSTRACT

Xylanases (EC 3.2.1.8) catalyze the breakdown of xylan, which is the second most abundant polysaccharide in plant cell walls. Biological catalysts have gained greater global attention than chemical catalysts in different industrial processes because they are highly selective, easy to control and have a negligible environmental impact. The aim of this study was to investigate the xylanolytic potential of white-rot fungi, optimize their physicochemical conditions and characterize the resulting xylanase. Sixty-eight white-rot fungus (WRF) isolates were screened for their xylanolytic potential and growth conditions for maximal xylanase production using cheap agricultural residue (wheat straw) as the sole carbon source. Five WRF isolates with high xylanase yields (73.63 ± 0.0283–63.6 ± 0.01247 U/ml) were selected by qualitative and quantitative screening methods. The optimum xylanase production occurred at pH 5.0 and 28 °C. Solid-state fermentation (SSF) yielded a high amount of xylanase. The highest xylanase activity (80.9–61.274 U/mL) was recorded in the pH range of 5.0–6.5 and at 50 °C. The metal ions Mg^{2+}, Ca^{2+} and Mn^{2+} enhanced the activity of xylanase (127.28–110.06 %), while $Cu^{2}+$, Fe^{2+} and K^{+} inhibited the activity with 43.4–17 % losses. The km and Vmax were 0.32–0.545 mg/mL and 86.95–113.63 μmol/min/mg, respectively. This finding indicates that wheat straw can be used for large-scale xylanase production under SSF conditions. The pH and temperature profiles and stabilities indicate that the xylanase produced in the present study can be applied in food and animal feed industries.

1. Introduction

The interest in the utilization of lignocellulosic biomass for the production of different biotechnological products and applications is increasing. The primary factors limiting the utilization of lignocellulosic biomass, such as crop residues, as sources of animal or human feedstuffs or chemical feedstocks are low digestibility, low protein content, high crude fibre content and low palatability due to its high fibrous content, such as cellulose, hemicelluloses and lignin [1].

In nature, there are efficient lignocellulosic fungi and bacteria that can produce several ligninolytic enzymes for the degradation of lignocellulosic wastes [2,3]. White-rot fungus (WRF) are among the lignocellulolytic microorganisms that can decompose and

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metabolize all plant cell constituents (cellulose, hemicellulose and lignin) via their enzymes [4]. This is due to their ability to produce relevant hydrolytic enzymes, including endo–1,4–D–glucanase, exo–1,4–D–glucanase and xylanase and oxidative extracellular enzymes [5].

Xylanases are a group of various enzymes involved in the breakdown of the complex structure of xylan into monosaccharaides and xylo-oligosaccharides [6]. Naturally, xylanase can be obtained from different sources, such as molluscs, insects, plants, animals and various microorganisms (bacteria, fungi, yeast, and algae) [7–11]. However, microorganisms have advantages over other sources due to their rapid multiplication in a short period of time, small space requirements, high potential for waste biomass biodegradation, production under controlled and closed systems and low production costs [12]. Xaylanase-producing microorganisms cover diverse ecological niches, such as marine and terrestrial habitats with decayed plant biomass, thermophilic and mesophilic environments, and the rumen of ruminants [8].

Based on molecular mass and isoelectric point, xylanase enzymes are categorized into two groups: (a) high-molecular weight enzymes with low isoelectric (acidic) points (HMWLI) and (b) low-molecular weight enzymes with high isoelectric (basic) points (LMWHI). However, this classification has drawbacks since it cannot describe all xylanases because not all xylanases belong to the (HMWLI) and (LMWHI) categories [13]. Therefore, as a solution to this drawback, more suitable systems, such as primary structures (crystals) and comparisons of catalytic domains, have been developed [14]. For more curated information on the characteristics and classification of enzymes, the carbohydrate-active enzyme (CAZy) database, which is involved in the breakdown, modification, and assembly of glycosidic bonds in carbohydrates and glycoconjugates, is appropriate. This database consists of genomic, sequence annotation, family classification, structural (3D crystal) and functional (biochemical) information from publicly available resources such as the National Center for Biotechnology Information (NCBI) [15]. The CAZy database (http://www.cazy.org) classifies xylanases into glycoside hydrolase (GH) families, which include 5, 7, 8, 9, 10, 11, 12, 16, 26, 30, 43, 44, 51 and 62. Among these families, the 16, 51 and 62 GH families have two catalytic domains with bifunctional properties, unlike the 5, 7, 8, 10, 11, and 43 GH families, which have a single distinct catalytic domain [14].

In recent years, the biotechnological use of xylanases has increased markedly. Xylanases are extensively used in the textile industry for fabric softening and to improve textile brightness [16,17]; in the paper industry to reduce lignin content and increase the brightness of the pulp [18]; and in the food industry to improve the organoleptic characteristics of fruit juices [19], decrease the firmness and increase the volume of the dough in the baking process [20]. Xylanases are also used as feed additives in animal feed to improve nutritive value by solubilizing and degrading insoluble polysaccharides [21]. Specifically, acidophilic xylanases have gained great attention for their applications in different industries, including the juice, animal feed, backing and brewing industries [22–24]. These factors make xylanase a very attractive enzyme for different industrial and biotechnological applications, such as the production of biofuel, bio pulping, bioleaching, and food and feed processing.

Several studies have been conducted to isolate new WRF strains with lignolytic potential for industrial application [25–27]. Since xylanase enzymes are required in large amounts for industrial level applications, there is a need to select potent microorganisms for xylanase production, followed by optimization of media components and culture conditions for maximum xylanase production. Therefore, the aim of this study was to screen WRF strains isolated from decayed woods under optimized media conditions for enhanced xylanase production and characterize these enzymes from isolates with high xylanolytic potential.

2. Materials and methods

2.1. Inoculum preparation and refreshment of fungal isolates

The sixty-eight white-rot fungi used in this study were obtained from the Microbial Biotechnology Research Laboratory, National Agricultural Biotechnology Research Center (NABRC), and were previously isolated from decaying woods and preserved on potato dextrose agar (PDA) slants at −20 °C. To refresh the isolates, potato dextrose agar (pH 5) plates were inoculated with 6 mm diameter plugs of white-rot fungal isolates and incubated at 28 °C for 7 days. Thereafter, the xylanolytic properties of 68 isolates were screened qualitatively and quantitatively.

2.2. Qualitative and quantitative screening of fungi for the production of xylanase

All 68 white rot fungal isolates were screened for their xylanolytic potential on Mandel and Reese agar media. The mineral salt media included the following (g/L): K_2HPO_4, 1.73; KH_2PO_4, 0.68; $(NH_4)_2SO_4$, 4; yeast extract, 1.4; $MgSO_4.7H_2O$, 0.1; NaCl, 4; $CaCl_2$, 0.02; $MnSO_4$, 0.01; and $FeSO_4$, 0.03. Agar, 20, was prepared and supplemented with 10.0 g/L birchwood xylan, and the pH of the media was adjusted to 5. The prepared medium was inoculated at the center of the Petri plate by taking a culture disk with a 5 mm plug from a 6-day-old culture plate and incubated at 28 °C for 7 days. Thereafter, the plates were stained by flooding with 10–15 mL of 1 % (w/v) Congo red solution for 15 min. The plates were destained by flooding with 1 M NaCl for 15 min, and the clear zone diameter was measured in cm using a digital ruler.

Seventeen white-rot fungal isolates with a high zone of hydrolysis were further screened via submerged state fermentation (SmF) for the production of xylanase. Five agar plugs of fungal mycelia were inoculated in Erlenmeyer flasks (250 mL) containing 100 mL of liquid mineral salt medium (MSM) and incubated at 28 °C in an orbital shaker (120 rpm) for 7 days. Then, the extracellular enzymes present in the culture filtrate were obtained by filtration through Whatman No. 1 filter paper. Thereafter, the culture filtrate was centrifuged at 10,000×*g* for 10 min, and the clear supernatant was used as a source of crude enzyme for further analysis.

2.3. Production of xylanase under SmF and SSF conditions using wheat straw as a carbon source

Wheat straw (WS) obtained from Holeta Agricultural Research Center (HARC) was dried in a hot-dry oven at 60 °C for 12 h and ground with a blender. The powder was sieved through a 1 mm mesh for SmF, whereas it was used without sieving for SSF.

Submerged fermentation was performed in duplicate in Erlenmeyer flasks (250 mL) containing 100 mL of MSM supplemented with 2 % WS as a carbon source (pH 5). After autoclaving at 121 °C for 20 min, the flasks were inoculated with 5 plugs of 7-day-old fungal mycelia. The inoculated flasks were incubated at 28 °C with shaking on an orbital shaker at 120 rpm for 7 days. The extracellular enzyme in the fermented samples was obtained by filtration through Whatman No. 1 filter paper followed by centrifugation of the filtrate at 10,000×*g* for 10 min. The clear supernatant was a source of crude enzyme for further analysis.

For solid-state fermentation, 10 g of nonsieved WS powder and 30 mL of MSM solution were mixed in an Erlenmeyer flask (250 mL) to achieve 70 % moisture content. Then, the flasks containing the fermentation medium were autoclaved at 121 °C for 20 min, inoculated with 5 disks of 7-day-old actively growing fungal isolates 6 mm in diameter and incubated at 28 °C for 10 days. After the end of the incubation period, the enzyme was extracted by a simple contact method [28]. The fermented samples were shaken (150 rpm) in 0.05 M sodium citrate buffer (pH 5.0) at a substrate-to-buffer ratio of 1:20 for 1 h at room temperature and then filtered through Whatman No. 1 filter paper. The filtrate was centrifuged at 10,000×*g* for 10 min at 4 °C to separate the enzyme from other components of the medium. The clear cell-free supernatant was used as a crude enzyme for determination of xylanase activity.

2.4. Xylanase assay and standard curve preparation

Xylanase activity was assayed as described by Bailey et al. [29] and Geweely [30] using 1 % birch wood xylan as a substrate in 0.05 M sodium citrate buffer (pH 5). Xylanase activity was assayed by incubating 2.25 mL of total reaction mixture containing 0.75 mL of diluted crude enzyme with buffer solution (1:100) and 1.5 mL of the substrate at 50 °C for 30 min. The enzyme blank was prepared by adding the same amount of enzyme to the reaction mixture after the reaction stopped. The reaction mixture containing the substrate was used as a reagent blank. The reaction was stopped by adding 2 mL 3,5–dinitrosalicylic acid (DNS) reagent followed by heating for 5 min at 100 °C and then rapid cooling by rinsing with cold water. After cooling, the absorbance was measured by UV spectrophotometry at 540 nm. A reagent blank was used to make the spectrophotometric reading zero, and the units of xylanase activity were determined from a xylose standard curve prepared as described previously [29]. The amount of xylose liberated was quantified using this regression equation, and one unit of xylanase activity (U) was defined as the amount of enzyme that liberated 1 μmol of reducing sugar (xylose) per min under standard assay conditions.

2.5. Optimization of culture conditions for xylanase production by WRFs

2.5.1. Effect of incubation temperature on xylanase production

To determine the effect of incubation temperature on xylanase production, MSM–wheat straw medium was inoculated with 7-day-old mycelia and incubated at 22, 25, 28, 31, 34, 37, 40, 43 and 46 °C for 7 days in a DRAWELL shaker incubator (DW–SI–211C) with a temperature accuracy of ±0.1 °C. All activities were measured in triplicate, and at the end of the incubation period, the enzyme was extracted as described in section 2.3, and the enzyme activity was measured under optimum conditions following the standard enzyme assay method.

2.5.2. Effect of pH on the production of xylanase

To determine the effect of pH on xylanase production, flasks containing 150 mL of MSM supplemented with 2 % WS were inoculated with five mycelial discs (6 mm) at different pH values (3.0, 4.0, 5.0, 6.0, 7.0, 8.0 and 9.0) at 28 °C for 7 days. Then, the enzyme was extracted as described in section 2.3, and the enzyme activity was assayed following the standard enzyme assay method.

2.5.3. Effect of carbon sources on the production of xylanase by WRF

The effects of different carbon sources (cellulose, lactose, carboxyl methyl cellulose (CMC) and xylan) on the production of xylanase for the selected fungal isolates were studied. The carbon sources supplemented with 1 % MSM were inoculated into 7-day-old mycelial discs (6 mm diameter) and incubated at 28 °C for 7 days at 120 rpm in an orbital shaker. After 7 days of incubation, the crude enzyme was extracted and assayed following the standard assay procedure.

2.5.4. Effect of nitrogen sources on xylanase production

The appropriate nitrogen sources for the maximum production of xylanase by the selected fungal isolates were determined by inoculating five mycelial discs (6 mm diameter) of 6-day-old culture in 150 mL of MSM supplemented with 0.2 % urea, yeast extract, ammonium sulfate and peptone. After incubation of the culture at 28 °C for 7 days, the crude enzyme was extracted, and its activity was determined following the standard assay procedure.

2.5.5. Production of xylanase under optimized production conditions

After determining the optimal and appropriate production parameters, the fermentation process was conducted by combining all the optimum parameters. Briefly, MSM was supplemented with 2 % WS as the sole carbon source and 0.2 % yeast extract as the nitrogen source at pH 5. Then, the media were inoculated with five mycelial discs (6 mm) of fungal mycelia and incubated at 28 °C for 7 days. Finally, the crude enzyme was harvested and subjected to partial purification.

2.6. Partial purification and protein content determination

A saturated ammonium sulfate solution was prepared as described by Wingfield [31] by dissolving 766.8 g of powdered $(NH_4)_2SO_4$ in 1 L of dH_2O. The precipitation was performed by diluting 100 mL of crude enzyme extract partially by gradually adding ammonium sulfate at saturations of 0–30 %, 30–40 %, 40–50 %, 50–60 %, 60–70 %, and 70–80 % (v/v). The suspension was stirred for 1 h and kept overnight at 4 °C. The sample was centrifuged at 3000×*g* for 10 min, after which the supernatant was discarded. The pellet was collected in an equal volume of the desired buffer of extract. Desalting was carried out against phosphate-buffered saline (PBS) (pH 8) using a dialysis membrane with a molecular weight cut-off of 10 kDa. The dialysis was carried out at room temperature by changing the buffer every 2 h three times, and then the dialysis continued at 4 °C overnight.

Protein content was measured according to the method of Lowry et al. [32] using the bovine serum albumin (BSA) standard curve. After the protein concentration was determined using the regression equation of the standard curve, the purity, specific activity and yield of the enzyme were determined as described previously [33].

2.7. Characterization of partially purified xylanase

2.7.1. Effect of temperature on xylanase activity and stability

The optimum catalytic temperature of partially purified xylanase was determined by assaying the enzyme activity in the range of 30–90 °C. The temperature stability was determined by measuring the residual xylanolytic activity after treatment at temperatures ranging from 50 to 90 °C for 60 min without the substrate, after which the enzyme assay was conducted under standard assay conditions.

2.7.2. Effect of pH on xylanase activity and stability

To determine the optimum pH of the partially purified enzyme, the assay was conducted using different buffers with pH values ranging from 3.5 to 9.5 and incubated at 50 °C for 30 min. The enzyme was mixed with buffers with varying pH values at a 1:10 (v/v) ratio. The buffers used were as follows: 0.1 M citrate buffer (pH 3.5–5.0), 0.2 M phosphate buffer (pH 6.0–7.5), and 0.2 M Tris–HCl buffer (pH 8–9.5) [34]. All the buffers were prepared according to the Gomori [35] method. For pH stability, the enzyme was pre-incubated without the substrate in different buffers with pH values ranging from 3.5 to 9.5 for 24 h at 4 °C, and thereafter, all the activities were determined under optimum pH and temperature conditions [36].

2.7.3. Effect of metal ions on partially purified xylanase activity

The effect of metal ions on xylanase activity was evaluated by incubating reaction mixtures containing 1.0 % birchwood xylan with K^+, Mn^{2+}, Mg^{2+}, Fe^{2+}, Ca^{2+} and Cu^{2+} at a concentration of 1.0 mM. The effects of different metal ions were studied using KCI, $MgSO_4$, $MnSO_4$, $CaCl_2$, $FeSO_4$, $ZnCl_2$ and $CuSO_4$. The degree of inhibition/activation of xylanase activity was expressed as the percentage of a control sample incubated in the absence of any additive.

2.7.4. Determination of xylanase activity under optimized physicochemical conditions

After characterization of the activity of the partially purified xylanase under different physico-chemical conditions, its activity was also determined using all the optimum parameters. Briefly, the assay was conducted by mixing 1.5 mL of 1 % birchwood xylan as a substrate in 0.05 M sodium citrate buffer (pH 5) with 0.75 mL of diluted (1:100) enzyme. Then, the reaction mixture was incubated at 50 °C for 30 min. After the end of the incubation period, 2 mL of DNS was added, and the mixture was boiled for 5 min. Finally, after cooling, the OD was measured at 540 nm using a UV spectrophotometer, and enzyme activity was determined using a regression equation from the xylose standard curve.

2.7.5. Determination of kinetic parameters

The kinetics parameters, Michaelis constant (km) and maximum velocity (Vmax) of the xylanase were determined by determining the enzyme activity at different birchwood xylan concentrations (0.1–1.2 mg/mL). The reaction mixture containing 0.75 ml of the enzyme solution and 1.5 mL of 0.1–1.2 mg/mL birchwood xylan in 50 mM citrate buffer (pH 5.0) was incubated for 30 min in a water bath at 50 °C. After incubation, the enzyme activity was measured, and the kinetic constants km and Vmax were estimated using the linear regression method of Lineweaver and Burk [37].

3. Data analysis

The experiments were conducted in triplicate with a completely randomized design (CRD). The experiments were evaluated with the R program (Rx64 3.6.3), and graphs were generated using the ggplot2 package in R. The results are expressed as the means ± standard deviations. Statistical comparisons were made by one-way analysis of variance (ANOVA), and means were separated by the least significant difference (LSD) test. Differences were considered significant when the *P* values were <0.05.

4. Results

4.1. Qualitative and quantitative screening of white-rot fungal isolates

Primary screening using Congo red staining revealed xylanolytic activity in all 68 fungal isolates as shown on [Fig. 1(a–e)]. However, the hydrolysis zones of the isolates ranged from 2.8 cm to 6.4 cm. Seventeen isolates produced distinct and high zones of hydrolysis that were greater than 4.55 cm around their colonies. These seventeen isolates were selected for further quantitative screening via submerged fermentation.

These isolates showed 12.84 U/mL to 72.53 U/mL xylanase activities (Table 1). Among the seventeen isolates, five (WR-39, WR.5, WR.68, WR.36 and WR.35) showed greater than 65.459 U/mL xylanase activity. The isolate WR.35 showed the highest xylanase activity (72.53 U/mL). Finally, the five isolates that showed maximum xylanase production were selected for SmF and SSF using wheat straw as the sole carbon source.

4.2. Production of xylanase under SmF and SSF conditions

All the isolates tested in the present study showed promising xylanase production potential both in SmF and SSF. The isolates showed xylanase activity ranging from 63.23 U/mL to 75.3 U/mL under SmF and from 68.7 U/mL to 87.99 U/mL under SSF. However, xylanase production in SSF was much greater than that in submerged fermentation (SmF). The xylanase activity of all the isolates except for WR.36 improved under SSF compared with the activity of the enzyme achieved in SmF (Fig. 2). Under SSF, WR.39 had the highest xylanase activity (87.99 ± 0.24 U/mL), followed by WR.5 and WR.35 (86.62 ± 0.23 and 84.42 ± 0.41 U/mL, respectively). WR.68 showed moderate improvement under SSF.

4.3. Optimization of culture conditions for xylanase production

4.3.1. Effect of incubation temperature on xylanase production

The results of this study indicated that incubation temperatures ranging from 25 to 31 °C favoured xylanase production for all the WRF isolates (WR.5, WR.35, WR.36, WR.39 and WR.68) (Fig. 3). The optimal temperature for all isolates to produce xylanase was 28 °C. All the isolates tested showed greater than 77 U/mL xylanase activity at 28 °C.

4.3.2. Effect of pH on xylanase production

The fungal isolates (WR.5, WR.35, WR.36, WR.39 and WR.68) exhibited xylanase production at pH values ranging from 5 to 6.5, with the highest xylanase production occurring at pH 5. The activity decreased rapidly below pH 5 and above pH 6.5 (Fig. 4).

4.3.3. Effect of different carbon sources

All the fungal isolates showed maximum xylanase production (75.031 ± 0.068–82.7 ± 0.757 U/mL) when xylan was used as the carbon source, followed by CMC and xylose, whose xylanase production profiles did not significantly differ ($P > 0.05$) (Table 2). While lactose achieved relatively moderate xylanase production, relatively low xylanase activity (<46.075 ± 0.89 U/mL) was detected in the medium with cellulose as the carbon source.

4.3.4. Effect of nitrogen sources

Among the nitrogen sources tested, yeast extract had the highest xylanase production (75.65 ± 1.03 U/mL), followed by ammonium sulfate (59.06 ± 1.89 U/mL) (Table 3). When fermentation medium was supplemented with urea, a relatively moderate amount of xylanase was produced in comparison to yeast extract. The lowest xylanase production was achieved by peptone (40.01 ± 0.94 U/mL). All the nitrogen sources had significantly different effects on xylanase production at $\alpha = 0.05$. An exceptional nonsignificant difference was detected between ammonium sulfate and urea in the case of the WR.39 isolate.

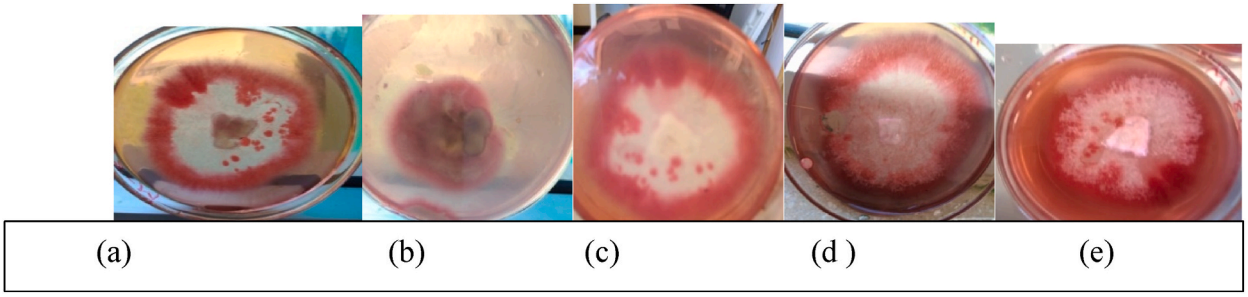

Fig. 1. The zone of hydrolysis produced by white-rot fungal isolates on Mandel and Reese agar media containing birchwood xylan (a) WR.68; (b) WR.35; (c) WR.36; (d) WR.5; (e) WR.39.

Table 1
Zone of hydrolysis (cm) of qualitatively screened and enzyme activity (U/mL) of quantitatively screened white-rot fungal isolates.

S/N	Isolates	Zone of hydrolysis (cm)	Enzyme activity (U/mL)
1	WR.68	6.40^{a}	69.93^{ab}
2	WR.36	6.15^{a}	70.70^{ab}
3	WR.39	6.10^{ab}	65.46^{c}
4	WR.5	5.75^{bc}	68.68^{b}
5	WR.35	5.60^{c}	72.53^{a}
6	WR.51	5.10^{d}	26.88^{d}
7	WR.10	4.85^{de}	21.01^{e}
8	WR.42	4.85^{de}	18.75^{efg}
9	WR.8	4.85^{de}	17.17^{fgh}
10	WR.45	4.80^{de}	12.84^{i}
11	WR.55	4.75^{de}	29.57^{d}
12	WR.33	4.65^{e}	16.20^{gh}
13	WR.11	4.60^{e}	17.65^{fgh}
14	WR.41	4.60^{e}	15.24^{hi}
15	WR.48	4.60^{e}	17.69^{fgh}
16	WR.7	4.60^{e}	18.75^{efg}
17	WR.2	4.55^{e}	19.23^{ef}

- White rot fungal isolates were grown on Mandel and Reese agar media supplemented with 1 % birichwood xylan as a carbon source.
- Quantitative screening was carried out under SmF on MSM-xylan broth media.
- The same superscript letters at the top right side within columns indicate that the values are not significantly different ($P < 0.05$).
- Least significant difference for qualitative (LSD) = 0.3829253 and for quantitative screening (LSD) = 2.947635.

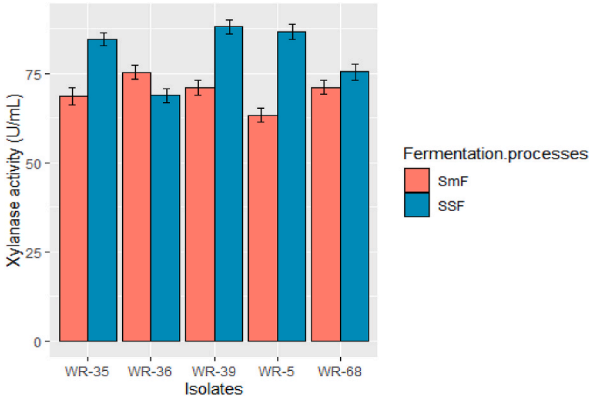

Fig. 2. Comparison of xylanase enzyme production under SmF and SSF by selected white-rot fungal isolates using wheat straw as a carbon source.

4.4. Comparative study of crude and partially purified xylanase enzyme activity

Xylanase from the WRF isolates showed maximum activity at 70 % ammonium sulfate saturation. Compared with unpurified xylanases, partially purified xylanases achieved relatively greater activity. The WR.36 and WR-39 isolates showed the highest xylanase activity after purification, followed by WR.5, which achieved a moderate improvement. However, xylanase from WR.35 and WR.68 showed slight improvement in xylanase activity after purification (Fig. 5).

4.5. Determination of protein concentration and specific activity

The protein content of the enzyme was estimated by using the Lowery method from the BSA standard curve. The protein concentration of the partially purified xylanase was determined from the regression equation ($Y = 0.5356x + 0.0123$) of the standard curve. The activity and specific activity of xylanase from all the isolates improved after 70 % ammonium sulfate precipitation. Partially purified xylanase enzymes from the WR.39 and WR.68 isolates exhibited the highest specific activity (86.42 and 86.68 U/mg) and 120.20 and 112.14 % yields, respectively, with 2.00- and 1.84-fold greater purities, respectively (Table 4).

4.6. Characterization of partially purified xylanase

4.6.1. Effect of pH on partially purified xylanase activity and stability

The results of this test indicated that the xylanase enzyme from all the WRF isolates had the highest activity in the pH range of 5–6.5. The maximum activity was observed at pH 5, except for xylanase obtained from WR.36, which showed the maximum activity at

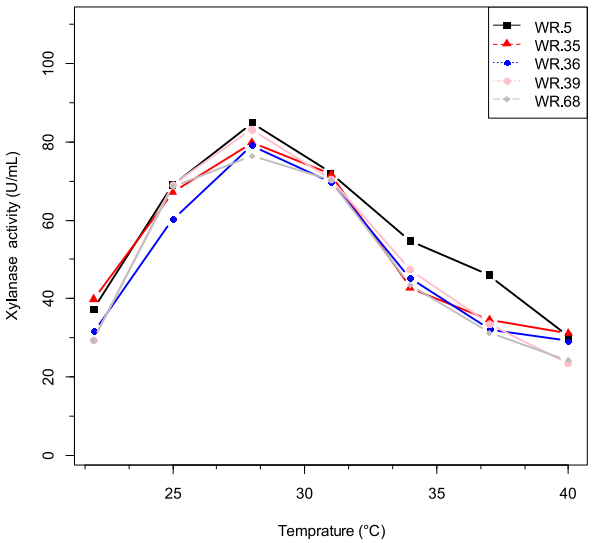

Fig. 3. Effect of temperature on xylanase production.

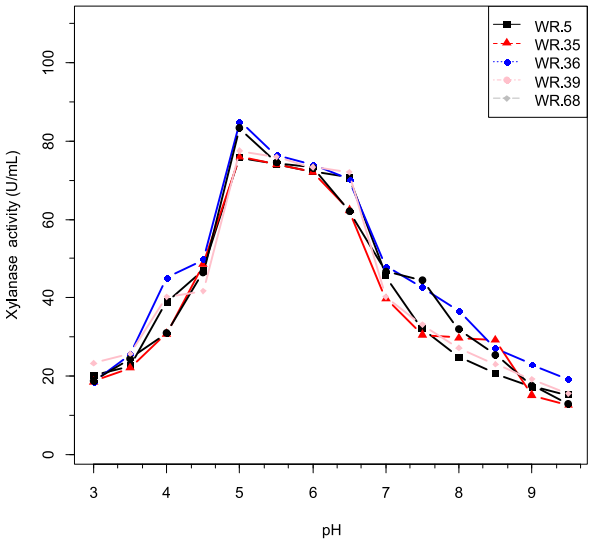

Fig. 4. Effect of pH on xylanase production.

Table 2
Effect of carbon source on xylanase production by the WRF isolates (the values are the means ± SDs of 3 replicates).

Xylanase activity (U/mL)					
Carbon sources	WR.5	WR.35	WR.36	WR.39	WR.68
Xylan	80.466 ± 0.827^{a}	75.031 ± 0.068^{a}	76.52 ± 1.2^{a}	82.7 ± 0.757^{a}	80.707 ± 2.6^{a}
CMC	74.36 ± 0.6^{b}	69.307 ± 0.245^{b}	71.42 ± 0.65^{b}	71.905 ± 1.246^{b}	73.97 ± 0.802^{b}
Xylose	73.4 ± 0.18^{b}	69.21 ± 0.235^{b}	70.606 ± 0.12^{b}	71.04 ± 0.235^{b}	73.63 ± 0.204^{b}
Lactose	58.4 ± 1.886^{c}	61.13 ± 1.80^{c}	68.345 ± 0.68^{c}	67.768 ± 0.136^{c}	67.816 ± 0.068^{c}
Cellulose	41.7 ± 0.36^{c}	38.76 ± 0.18^{d}	30.68 ± 1.88^{d}	46.075 ± 0.89^{d}	44.824 ± 0.18^{d}

- The same superscript letters on the top right side within columns indicate that the values are not significantly different, whereas different subscript letters indicate that the values are significantly different ($P < 0.05$).
- A pairwise group comparison of different carbon sources was conducted by the Fisher-LSD test.

Table 3
Effect of nitrogen source on xylanase production by WRF isolates (values are the means ± SDs of 3 replicates).

Xylanase activity (U/mL)					
Nitrogen sources	WR.5	WR.35	WR.36	WR.39	WR.68
Yeast extract	74.89 ± 0.44^{a}	75.46 ± 0.544^{a}	75.66 ± 1.03^{a}	74.45 ± 2.65^{a}	72.58 ± 0.93^{a}
Ammonium sulfate	59.06 ± 1.89^{b}	62.14 ± 1.533^{b}	69.02 ± 0.51^{b}	60.02 ± 0.84^{b}	59.50 ± 2.78^{b}
Urea	52.81 ± 1.65^{c}	54.30 ± 0.47^{c}	59.93 ± 1.64^{c}	56.20 ± 0.12^{b}	54.4 ± 2.20^{c}
Pepton	52.81 ± 1.43^{d}	42.85 ± 2.45^{d}	40.01 ± 0.94^{d}	43.62 ± 1.87^{c}	46.315 ± 1.88^{d}

- The same superscript letters on the top right side within columns indicate that the values are not significantly different, whereas different subscript letters indicate that the values are significantly different at $P < 0.05$.
Pairwise group comparisons of different nitrogen sources were conducted by the Fisher-LSD test

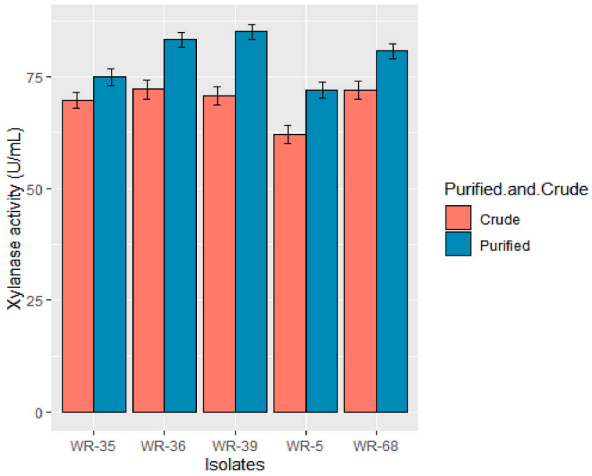

Fig. 5. Comparative study of crude and partially purified xylanase enzymes.

Table 4
Summary of the crude and partially purified xylanase enzymes.

	Activity (U/mL)		Total protein (mg/mL)		Specific activity (U/mg)		Yield (%)	Purity
Isolates	Crude	Purified	Crude	Purified	Crude	Purified		
WR.5	62.09	71.95	1.45	0.95	42.82	76.01	115.88	1.77
WR.35	69.74	74.98	2.33	1.30	29.88	57.50	107.52	1.92
WR.36	72.14	83.26	2.46	1.84	29.29	45.32	115.40	1.55
WR.39	70.75	85.04	1.64	0.98	43.14	86.42	120.20	2.00
WR.68	72.01	80.75	1.53	0.93	47.07	86.68	112.14	1.84

pH 5.5. Xylanase enzyme activity in all the WRF isolates decreased on either side of the optimum range from acidic to neutral pH and alkaline pH (Fig. 6a).

According to the results of this test, xylanase enzymes from all the WRF isolates retained more than 60 % of their residual activity in the pH range of 5–7. However, xylanase from each fungal isolate exhibited maximum residual activity at different pH values. For example, xylanases from WR.5, WR.39 and WR.68 exhibited maximum residual activity (87.065 %, 82.5 % and 86.816 %, respectively) at pH 6. However, xylanases from WR.35 and WR.36 retained 87.726 % and 96.535 % of their residual activity, respectively, at pH 6.5 (Fig. 6b).

4.6.2. Effect of temperature on partially purified xylanase activity and stability

The results indicated that the xylanases from all the isolates exhibited good activity in the temperature range of 50–60 °C, but the maximum xylanase activity (79.17, 74.9, 74.84, 77.82 and 80.034 U/ml) was observed for WR.5, WR.35, WR.36 and WR.68, respectively, at 50 °C. The enzyme activity decreased below 50 °C and above 60 °C (Fig. 7a).

The thermostability test showed that xylanases from all the isolates retained more than 90 % and 80 % of their residual activity at 30 and 40 °C, respectively. The residual activities for all xylanases were approximately 60 % at 50 °C, except for WR.39 and WR.68, which retained 50 % of their activity at 60 °C (Fig. 7b).

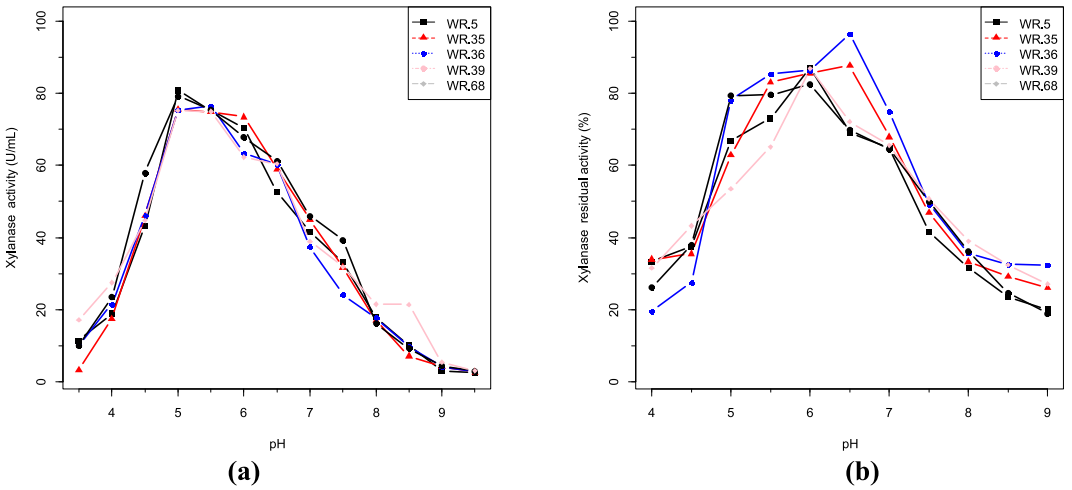

Fig. 6. Effect of pH on partially purified xylanase activity (a) and stability (b).

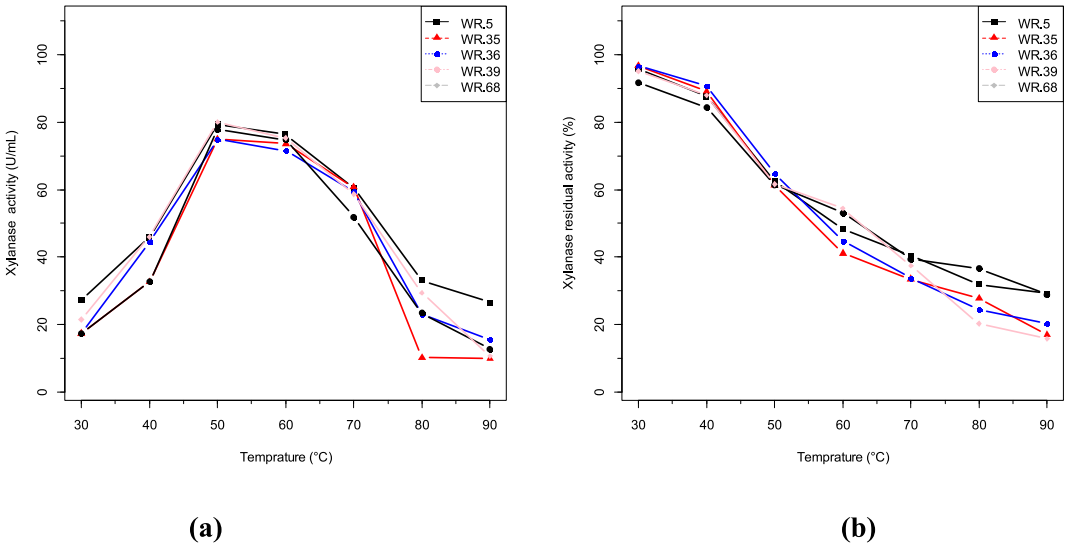

Fig. 7. Effect of temperature on partial purified xylanase activity (a) and stability (b).

4.6.3. Effect of metal ions on partially purified xylanase

Among the metal ions tested in this study, Mg^{2+}, Ca^{2+} and Mn^{2+} enhanced the activity of xylanase (Fig. 8). The incorporation of these metals enhanced the relative activity of partially purified xylanase by 10–27 %. The inhibition of enzyme activity was observed in the presence of Cu^{2+} and $K^{+,}$ which caused an ~40 % loss of xylanase activity in all the isolates. Fe^{2+} had a relatively slight inhibitory effect, causing an ~20 % decrease in enzyme activity.

4.7. Determination of kinetic parameters

The Vmax and km were calculated from the regression equation of Lineweaver–Burk plots. The regression coefficients (R^2) were 0.9912, 0.992, 0.9894, 0.9955 and 0.9964 for WR.5, WR.35, WR–36, WR–39 and WR–68, respectively (Fig. 9). The Vmax and km for xylanases from WR.5, WR.35, WR.36, WR.39 and WR.68 were 106.38, 113.63, 86.95, 109.89 and 99 μmol min^{-1} mg^{1}, respectively, and 0.415, 0.545, 0.339, 0.527 and 0.32 mg/mL, respectively.

5. Discussion

Xylanolytic enzymes have gained great attention because they have potential roles in biorefinery processes as well as industrial applications in various fields, such as wine making, textile, paper and pulp industries, animal feed industries, bread making, and fruit

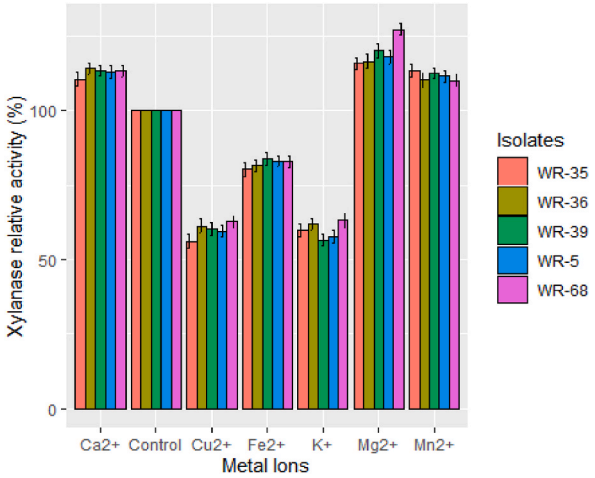

Fig. 8. Effect of metal ions on the activity of partially purified xylanase.

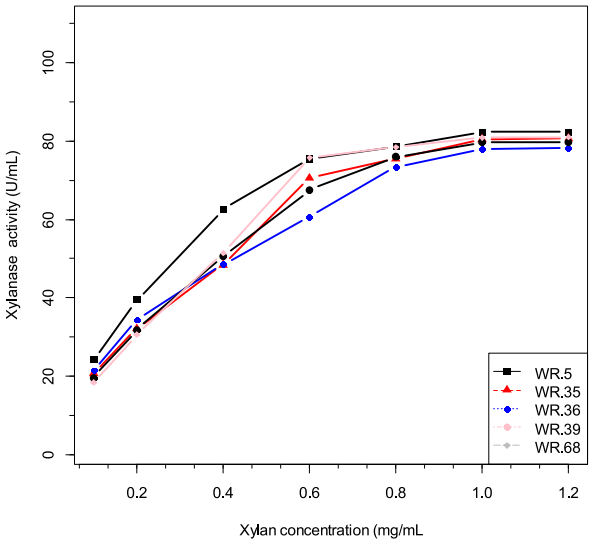

Fig. 9. Michael–Menten plot of xylanase enzymes from selected WRF isolates.

juice extraction [38,39]. In line with this, in the present study, an attempt was made to isolate and characterize xylanase from WRF to determine its potential applications.

All the WRF isolates screened in this study showed xylanolytic potential with various hydrolysis zones. The appearance of a clear zone around the colony implies that the isolates have the potential to convert xylan to xylose due to the hydrolytic action of xylanases. Among the 17 isolates that were selected based on their zone of hydrolysis and subjected to quantitative screening, five showed maximum xylanase activity, indicating the potential of WRF for the production of xylanase for various applications.

The results of the present study indicate that the pH of the fermentation medium in which the WRF isolates showed good growth and xylanase production ranged from 5 to 6.5. However, all the isolates showed the highest xylanase production at pH 5. This finding agrees with previous reports of maximum xylanase production at pH 5 from white-rot fungi [40]. Ramanjaneyulu et al. [41] reported that xylanase production increased with increasing initial pH of the medium from 4.0 to 5.5, with a maximum yield of 5.0. This may be because acidic pH (5.0–6.5) is the optimum pH for the growth of WRF isolates and consequently favours xylanase production.

In this study, an incubation temperature of 28 °C maximized the yield of xylanase. The amount of xylanase decreased below 25 °C and above 31 °C. Similarly, Guan et al. [34] reported that temperatures ranging from 28 °C to 30 °C favoured the production of xylanase from *Cladosporium oxysporum*. A higher xylanase yield at a lower temperature range (28–31 °C) is advantageous since it reduces the energy requirement and facilitates the production of xylanase without the need for a specialized incubation instrument. This suggests that the WRF isolates obtained in this study can be used for xylanase production at ambient temperature, reducing the cost of energy and specialized incubation facilities.

In this investigation, supplementation of the medium with a nitrogen source significantly influenced xylanase production with a 95

% confidence level. The maximum amount of xylanase was obtained when yeast extract was supplemented as a nitrogen source. This observation is in line with the findings of Ramanjaneyulu et al. [41], who reported that yeast extract yielded the greatest amount of xylanase xylan. Similarly, previous reports revealed that *Cladosporium oxysporum*, *a* white rot fungus, and *Penicillium oxalicum* produce high amounts of xylanase when yeast extract is applied to growth media [34,42]. This stimulating effect of yeast extract on xylanase production is due to increased biomass production, as it is a vital nutrient for microbial growth.

The WRF isolates produced the greatest amount of xylanase when xylan was used as the carbon source. The WRF isolates in the present study could be utilized for the production of xylanases from lignocellulosic biomass and pretreatment of lignocellulosic biomass for the production of fermentable sugars for various industrial applications. Similarly, (Rosmine et al. [43] reported that xylan was the best substrate for xylanase production out of different carbon sources tested for xylanase production.

All isolates except WR–36 yielded more xylanase under SSF conditions than under SmF conditions. This is in agreement with previous reports that revealed the highest amount of xylanase production by different fungi under SSF compared to that under SmF [44–46]. The increased yield of xylanase produced by the isolate observed in SSF may be due to close contact between the mycelium and substrate, which is not possible during SmF. This may also be due to the similarities in environmental conditions between the SSF and the natural white-rot fungal habitat (wood and organic material).

In the present study, the partially purified xylanase from all the isolates showed maximum xylanase activity and specific activity. This may be because ammonium salt precipitation removed other contaminants and increased the enzyme concentration. Several earlier findings [34,47,48] indicated that the purification of xylanase by the ammonium precipitation method improved its activity, specific activity, yield (%) and purification.

The optimum pH for xylanase activity ranged from 5 to 6.5 (acidic conditions), and the enzyme was stable in the pH range of 5–7. Most of the xylanase activity decreased below and above the optimum pH. This is because changes in the pH outside the optimum range cause enzymes to denature since they are proteins. Enzymes are sensitive to changes in the hydrogen ion concentration that cause denaturation by altering the degree of ionization of an enzyme's acidic and basic side groups located in the active site. There are several commercially available fungal xylanases (Bioxylanase, Allzyme PT, Gammazym X400OL and Solvay Pentosanase) that exhibited similar optimal pH and temperature as the xylanase enzyme produced in this study and used in the animal feed, starch, baking and brewing industries [24]. There are also recent findings in line with this study. Adigüzel and Tunçer [23] reported that partially purified xylanase with an optimal pH of 6 and a pH of 4–9 with >54 % activity retention enhanced orange and grape juice clarity and improved the dough volume. Similarly, da Silva et al. [22] reported that an endo-xylanase with optimal activity at a range of pH values from 5.0 to 6.0 enhanced the clarity of mango, banana and tangerine juices. Therefore, the enzymes produced in this study from the WRF isolates showed characteristics suitable for application in the animal feed upgrading, starch producing, baking, brewing and fruit juice producing industries.

In this study, the thermostability of partially purified xylanases varied widely (90%–50 %). This result indicated that xylanases could be used in food processing, such as fruit juice clarification, bread making and animal feed industries, since most commercially available xylanases used in these industries have optimum temperatures ranging from 40 to 60 °C. Adigüzel and Tunçer [1] reported that partially purified xylanase from the *Streptomyces species* AOA40 increased the juice clarity of grape juice at 50 °C and of orange juice at 60 °C, improved the dough volume and modified the texture of the fresh bread crumb. da Silva et al. [22] reported that the application of partially purified endo-xylanase at an optimum temperature of 55 °C improved the juice quality of mango, banana and tangerine juices.

The metal ions Mg^{2+}, Ca^{2+} and Mn^{2+} enhanced the relative activity of xylanase, while Cu^{2+}, K^{+} and Fe^{2+} inhibited this activity. The inhibitory or stimulatory effects of metal ions on enzyme activity may be due to their interactions with the sulfhydryl or carboxyl groups of the enzyme, which destabilize its molecular structure and lead to conformational changes and subsequently cause inactivation or activation of the enzyme. Similarly, Ping et al. [49] revealed that Mg^{2+}, Mn^{2+}, and Ca^{2+} enhanced xylanase activity.

In the present study, the WRF isolates WR.68 and WR.36, which had the smallest km values (0.32 mg/mL and 0.34 mg/mL, respectively), had relatively high affinities for xylan. This indicates that the WRF isolates WR.68 and WR.36 have potential for the production of biotechnologically important biomolecules from lignocellulosic biomass.

6. Conclusion

White-rot fungal isolates from decaying wood can be good xylanase sources. Based on the results obtained from production optimization, it can be concluded that using a cheap agricultural residue (wheat straw) yielded a high amount of xylanase under SSF conditions. Therefore, wheat straw can be used for large-scale xylanase production under SSF conditions. The pH and temperature profiles and stabilities indicate that xylanase can be suitable for application in the food industry (fruit juice and bakery), animal feed industry and wine industry. The lower km value of the xylanase enzyme indicates that the enzyme has the highest affinity for the substrate (xylan). This implies that a small quantity of xylanase will hydrolyse a considerably high amount of xylan into its monomer.

Data availability statement

Has data associated with your study been deposited into a publicly available repository?

- No
- Data included in article/sup. Material/referenced in article

CRediT authorship contribution statement

Tariku Abena: Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Addis Simachew:** Writing – review & editing, Visualization, Supervision.

Declaration of competing interest

We wish to confirm that there are no known conflicts of interest associated with this publication and there has been no significant financial support for this work that could have influenced its outcome. We confirm that the manuscript has been read and approved by all named authors and that there are no other persons who satisfied the criteria for authorship but are not listed. We further confirm that the order of authors listed in the manuscript has been approved by all of us.

We confirm that we have given due consideration to the protection of intellectual property associated with this work and that there are no impediments to publication, including the timing of publication, with respect to intellectual property. In so doing we confirm that we have followed the regulations of our institutions concerning intellectual property. We further confirm that any aspect of the work covered in this manuscript has not involved experimental animals or human.

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References

[1] R.B. Melati, F.L. Shimizu, G. Oliveira, F.C. Pagnocca, W. de Souza, C. Sant'Anna, M. Brienzo, Key factors affecting the recalcitrance and conversion process of biomass, BioEnergy Research 12 (2019) 1–20.
[2] C. Sanchez, Lignocellulosic residues: biodegradation and bioconversion by fungi, Biotechnol. Adv. 27 (2009) 185–194.
[3] R. Renugadevi, M.P. Aryyappadas, P.H. Preethy, S. Savetha, Isolation, screening and induction of mutation in strain for extracellular lignin peroxidase producing bacteria from soil and its partial purification, J. Res. Biol. 4 (2011) 312–318.
[4] T.H. De Koker, J. Zhao, S.F. Allsop, B.J.H. Janse, Isolation and enzymic characterization of South African white-rot fungi, Mycol. Res. 104 (2000) 820–824.
[5] V. Elisashvili, M. Penninckx, E. Kachlishvili, N. Tsiklauri, E. Metreveli, T. Kharziani, G. Kvesitadze, Lentinus edodes and Pleurotus species lignocellulolytic enzymes activity in submerged and solid-state fermentation of lignocellulosic wastes of different composition, Bioresour. Technol. 99 (2008) 457–462.
[6] N. Bhardwaj, B. Kumar, K. Agarwal, V. Chaturvedi, P. Verma, Purification and characterization of a thermoacid/alkali stable xylanases from *Aspergillus oryzae* LC1 and its application in Xylo-oligosaccharides production from lignocellulosic agricultural wastes, Int. J. Biol. Macromol. 122 (2019) 1191–1202.
[7] M. Wainø, K. Ingvorsen, Production of ß-xylanase and ß-xylosidase by the extremely halophilic archaeon Halorhabd usutahensis, Extremophiles 7 (2003) 87–93.
[8] S. Singh, G.K. Sidhu, V. Kumar, D.S. Dhanjal, S. Datta, J. Singh, Fungal xylanases: sources, types, and biotechnological applications, in: Recent Advancement in White Biotechnology through Fungi, Springer, Cham, 2019, pp. 405–428.
[9] Y. Brennan, W.N. Callen, L. Christoffersen, P. Dupree, F. Goubet, S. Healey, M. Hernández, M. Keller, K. Li, N. Palackal, A. Sittenfeld, Unusual microbial xylanases from insect guts, Appl. Environ. Microbiol. 70 (2004) 3609–3617.
[10] J.K. Jensen, M. Busse-Wicher, C.P. Poulsen, J.U. Fangel, P.J. Smith, J.Y. Yang, M.J. Peña, M.H. Dinesen, H.J. Martens, M. Melkonian, G.K.S. Wong, Identification of an algal xylan synthase indicates that there is functional orthology between algal and plant cell wall biosynthesis, New Phytol. 218 (2018) 1049–1060.
[11] M.V. Sizova, J.A. Izquierdo, N.S. Panikov, L.R. Lynd, Cellulose-and xylan degrading thermophilic anaerobic bacteria from biocompost, Appl. Environ. Microbiol. 77 (2011) 2282–2291.
[12] M. Mitreva-Dautova, E. Roze, H. Overmars, L. de Graaff, A. Schots, J. Helder, A. Goverse, J. Bakker, G. Smant, A symbiont-independent endo-1, 4-ß-xylanase from the plant-parasitic nematode Meloidogyne incognita, Mol. Plant Microbe Interact. 19 (2006) 521–529.
[13] T. Collins, M.A. Meuwis, I. Stals, M. Claeyssens, G. Feller, C. Gerday, A novel family 8 xylanase, functional and physicochemical characterization, J. Biol. Chem. 277 (2002) 35133–35139.
[14] T. Collins, C. Gerday, G. Feller, Xylanases, xylanase families and extremophilic xylanases, FEMS Microbiol. Rev. 29 (2005) 3–23.
[15] V. Lombard, Ramulu H. Golaconda, E. Drula, P.M. Coutinho, B. Henrissat, The carbohydrate-active enzymes database (CAZy) in 2013, Nucleic Acids Res. 42 (2014) 490–495.
[16] S.M. Atalla, N.E. Ahmed, H.M. Awad, N.G. El Gamal, A.R. El Shamy, Statistical optimization of xylanase production, using different agricultural wastes by Aspergillus oryzae MN894021, as a biological control of faba bean root diseases, Egyptian Journal of Biological Pest Control 30 (2020) 1–12.
[17] M.L.T.M. Polizeli, C.S. Rizzatti, R. Monti, H.F. Terenzi, J. Jorge, D.S. Amorim, Xylanases from fungi: properties and industrial applications, Appl. Microbiol. Biotechnol. 67 (2005) 577–591.
[18] V. Kumar, P. Shukla, Functional aspects of xylanases toward industrial applications, in: P. Shukla (Ed.), Frontier Discoveries and Innovations in Interdisciplinary Microbiology, Springer, Berlin, 2015, pp. 157–165.
[19] L. Kumar, S. Nagar, A. Mittal, N. Garg, V.K. Gupta, Immobilization of xylanase purified from *Bacillus pumilus* VLK-1 and its application in enrichment of orange and grape juices, J. Food Sci. Technol. 51 (2014) 1737–1749.
[20] Z. Jiang, X. Li, S. Yang, L. Li, S. Tan, Improvement of the bread making quality of wheat flour by the hyperthermophilic xylanase B from Thermotoga maritime, Food Res. Int. 38 (2005) 37–43.
[21] G.K. Goswami, R.R. Pathak, Microbial xylanases and their biomedical applications: a review, Int. J. Basic Clin. Pharmacol. 2 (2013) 237–246.
[22] P.O. da Silva, N.C. de Alencar Guimarães, J.D.M. Serpa, D.C. Masui, C.R. Marchetti, N.V. Verbisck, F.F. Zanoelo, R. Ruller, G.C. Giannesi, Application of an endo-xylanase from *Aspergillus japonicus* in the fruit juice clarification and fruit peel waste hydrolysis, Biocat.Agri. Biotechnol. 21 (2019) 101312.
[23] A.O. Adigüzel, M. Tunçer, Production, characterization and application of a xylanase from Streptomyces species AOA40 in fruit juice and bakery industries, Food Biotechnol 30 (2016) 189–218.
[24] D. Haltrich, B. Nidetzky, K.D. Kulbe, W. Steiner, S. Župančič, Production of fungal xylanases, Bioresour. Technol. 58 (1996) 137–161.
[25] B.S.S. Kumari, K.D. Kumar, K.Y. Usha, A. Ramya, B.R. Reddy, Secretion of ligninolytic enzymes by the white rot fungus *Stereum ostrea* on saw dust under the influence of chlorpyrifos, IJCRT 6 (2018) 2320–2882.
[26] Y. Saito, H. Tsuchida, T. Matsumoto, Y. Makita, M. Kawashima, J. Kikuchi, M. Matsui, Screening of fungi for decomposition of lignin- derived products from Japanese cedar, J. Biosci. Bioeng. 20 (2018) 1–7.
[27] Y. Su, X. Yu, Y. Sun, G. Wang, H. Chen, Evaluation of screened lignin-degrading fungi for the biological pretreatment of corn stover, Sci. Rep. 8 (2018) 5385.
[28] C. Krishna, M. Chandrasekaran, Banana waste as substrate for a-amylase production by *Bacillus subtilis* BTK (106) solid-state fermentation, Appl. Microbiol. Biotechnol. 46 (1996) 106–111.
[29] M.J. Bailey, P. Biely, K. Poutanen, Interlaboratory testing of methods for assay of xylanase activity, J. Biotechnol. 23 (1992) 257–270.
[30] N.S. Geweely, Purification and characterization of acido-thermophilic xylanase from *spergillus. A.J.B.A.S* 5 (2011) 214–219.

[31] P. Wingfield, Protein precipitation using ammonium sulfate. Current protocols in protein science, Appendix 3 (2001), https://doi.org/10.1002/0471140864.psa03fs13. Appendix–3F.
[32] O.H. Lowry, N.J. Rosebrough, A.L. Farr, R.J. Randall, Protein measurement with the Folin phenol reagent, J. Biol. Chem. 193 (1951) 265–275.
[33] Guide to protein purification, in: R.R. Burgess, M.P. Deutscher (Eds.), Methods in Enzymology, second ed. vol. 436, Academic Press, San Diego, CA, 2009.
[34] G.Q. Guan, P.X. Zhao, J. Zhao, M.J. Wang, S.H. Huo, F.J. Cui, J.X. Jiang, Production and partial characterization of an alkaline xylanase from a novel fungus Cladosporium oxysporum, BioMed Res. Int. 2016 (2016).
[35] G. Gomori, Preparation of buffers for use in enzyme studies, Methods Enzymol. (1955) 138–146.
[36] L.C. de Oliveira Simões, R.R. da Silva, C.E. Maurício Boscolo de Oliveira Nascimento, Eleni Gomes, Roberto da Silva, Purification and physicochemical characterization of a novel thermostable xylanase secreted by the fungus *Myceliophthora heterothallica* F.2.1.4, Appl. Biochem. Biotechnol. 188 (2019) 991–1008.
[37] H. Lineweaver, D. Burk, The determination of enzyme dissociation constants, J. Am. Chem. Soc. 56 (1934) 658–666.
[38] B.S. Chadha, B. Kaur, N. Basotra, A. Tsang, A. Pandey, Thermostable xylanases from thermophilic fungi and bacteria: current perspective, Bioresour. Technol. 277 (2019) 195–203.
[39] B. Sunkar, B. Kannoju, B. Bhukya, Optimized production of xylanase by penicillium purpurogenum and ultrasound impact on enzyme kinetics for the production of monomeric sugars from pretreated corn cobs, Front. Microbiol. 11 (2020) 772.
[40] R. Saleem, M. Khurshid, S. Ahmed, Xylanase, Laccase and Manganese Peroxidase Production from White Rot Fungi, vol. 5, I.J.E.E., 2014, pp. 59–66.
[41] G. Ramanjaneyulu, A. Sridevi, P. Seshapani, A. Ramya, K.D. Kumar, G.P.K. Reddy, B.R. Reddy, Enhanced production of xylanase by Fusarium sp. BVKT R2 and evaluation of its biomass saccharification efficiency, 3 Biotech 7 (2017) 1–17.
[42] R. Muthezhilan, R. Ashok, S. Jayalakshmi, Production and optimization of thermostable alkaline xylanase by *Penicillium oxalicum* in solid-state fermentation, Afr. J. Microbiol. Res. 1 (2007) 20–28.
[43] E. Rosmine, Neethu Changan Edassery Sainjan, Reshma Silvester, Saramma Aikkarakunnath Varghese, Screening and characterization of xylanase producing aquatic *streptomyces* species from the sediments of cochin backwater system, J.Bio.Innov 7 (2018) 475–502.
[44] J. Liu, J. Yang, R. Wang, L. Liu, Y. Zhang, H. Bao, J.M. Jang, E. Wang, H. Yuan, Comparative characterization of extracellular enzymes secreted by Phanerochaete chrysosporium during solid-state and submerged fermentation, Int. J. Biol. Macromol. 152 (2020) 288–294.
[45] G. Fikiru, A. Melaku, K. Ameha, Production, purification and characterization of xylanase from oyster mushroom (pleurotus species), J. Nat. Sci. Res. 6 (2016) 2016.
[46] S. Agnihotri, D. Dutt, C.H. Tyagi, A. Kumar, J.S. Upadhyaya, Production and biochemical characterization of a novel cellulase-poor alkali-thermotolerant xylanase from *Coprinellus disseminatus* SW-1 NTCC 1165, World J. Microbiol. Biotechnol. 26 (2010) 1349–1359.
[47] A. Gautam, A. Kumar, A.K. Bharti, D. Dutt, Rice straw fermentation by *Schizophyllum commune* ARC-11 to produce high level of xylanase for its application in prebleaching, J. Genet. Eng. Biotechnol. 16 (2018) 693–701.
[48] R.A. Deshmukh, S. Jagtap, M.K. Mandal, S.K. Mandal, Purification, biochemical characterization and structural modelling of alkali-stable β-1,4-xylan xylanohydrolase from *Aspergillus fumigatus* R1 isolated from soil, BMC Biotechnol. 16 (2016) 1–12.
[49] L. Ping, M. Wang, X. Yuan, F. Cui, D. Huang, W. Sun, B. Zou, S. Huo, H. Wang, Production and characterization of a novel acidophilic and thermostable xylanase from *Thermoascus aurantiacu*, Int. J. Biol. Macromol. 109 (2018) 1270–1279.