Journal of Advances in Biology & Biotechnology

24(12): 1-12, 2021; Article no.JABB.81968
ISSN: 2394-1081

Purification and Characterization of Thermostable Alkaline Xylanase by *Aspergillus terreus* from Elephant Dung

Richa Nenava [a], Sadhana Nighojkar [b], Mukesh Patidar [c], Anil Kumar [a*] and Anand Nighojkar [c]

[a] School of Biotechnology, Devi Ahilya University, Khandwa Rd., Indore 452001, India.
[b] Mata Gujri College of Professional Studies, A.B. Road, Indore 452001, India.
[c] Maharaja Ranjit Singh College of Professional Sciences, Hemkunt Campus, Khandwa Road, Indore 452001, India.

Authors' contributions

This work was carried out in collaboration between all authors. Author RN carried the experimental work and wrote the first draft of the manuscript. Author MP investigated the software analysis and validation of data and investigated studies and managed literature search. Author AN designed the study, supervised the experimental work, prepared the figures and checked manuscript. Author SN also, designed the experiment, checked and corrected the first draft of the manuscript. Author AK designed the experimental work and finally corrected the entire manuscript. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/JABB/2021/v24i1230254

Open Peer Review History:
This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: https://www.sdiarticle5.com/review-history/81968

Original Research Article

Received 20 October 2021
Accepted 24 December 2021
Published 25 December 2021

ABSTRACT

Aims: Purification and characterization of xylanase by Aspergillus *terreus* isolated from elephant dung.
Study Design: Xylanase enzyme produced by A. *terreus* was extracted from the fermented solid medium using solid state fermentation and purified using chromatographic techniques. The purified enzyme was characterized for physio-chemical and kinetic properties.
Place and Duration of Study: Experiments were performed at the School of Biotechnology, Devi Ahilya University, Indore, INDIA and Maharaja Ranjit Singh College of Professional Sciences, Indore, INDIA, between June, 2019 and September, 2021.
Methodology: The enzyme was extracted and purified using ammonium sulphate precipitation, ion

**Corresponding author: E-mail: ak_sbt@yahoo.com;*

exchange chromatography using DEAE cellulose and gel filtration chromatography (GFC) using Sephadex G-200. The molecular weight of the purified enzyme was determined using native poly acrylamide gel electrophoresis (Native PAGE) and sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE). The purified enzyme was characterized to determine pH and temperature optima. Thermostability, pH stability and substrate kinetics were studied for purified xylanase. Effect of several metal ions and detergents were also studied.

Results: The alkali-thermo xylanase of Aspergillus *terreus* was purified to 14.6-fold with a 39.48% recovery through IEC on DEAE-cellulose and GFC using Sephadex G-200. The purified enzyme had a specific activity, 111.6 U/mg. The SDS-PAGE revealed that the enzyme was monomeric with a molecular weight of 87 kDa. The optimum pH and temperature were 8 and 60^0 C, respectively. The Km and Vmax values of the partially purified xylanase were found to be 0.016 mg/l and 102.64 U/mg protein, respectively with birch wood xylan as substrate.

Conclusion: The enzyme was active over a wide pH range of 6-11 and temperature range of 40^0 C to 80°C, indicating its alkali tolerant and thermostable characteristics which is the requirement of different industries. Several ions namely Zn^{2+} enhanced xylanase activity by 187% followed by Ca^{2+} (129%), Mg^{2+} (123%), NH_4^+ (146%) and Fe^{3+} (118%) at 10 mM concentration. However, Cu^{2+} and Hg^{2+} completely inhibited xylanase activity. This research provides the basis for application of xylanase and improves its biotechnological potential in mainly in paper pulp industry, biofuel generation and in bread baking.

Keywords: Aspergillus terreus; purification; DEAE- cellulose; thermostable; alkaline; xylanase; characterization

1. INTRODUCTION

Xylan consist of β-1,4 -linked D-xylopyranose units organized in a homo polymeric main chain carrying short side chains consisting of O-acetyl, α-L -arabinofuranosyl and α-D glucuronyl residues. [1,2]. Xylan are the second most plentiful polysaccharide existing on earth [3]. Depolymerization of xylan into simple sugars and xylooligosaccharides is completed by xylanase involving a cluster of enzymes such as endo1,4-β-d-xylanases (EC 3.2.1.8), β-d-xylosidases (E.C. 3.2.1.37), α-glucuronidase (EC 3.2.1.139) acetylxylan esterase (EC 3.1.1.72), α-l-arabinofuranosidases (E.C. 3.2.1.55), p-coumaric esterase (3.1.1.B10) and ferulic acid esterase (EC 3.1.1.73) [4,5] (Gomez et al. 2008; Juturu and Wu 2014). Xylanases are broadly rich in nature; they are produced by bacteria, fungi, protozoa, algae, gastropods, arthropods, nematodes, etc. [6]. Microbial xylanase involves significant research interest in recent years because of their potential application in the food, animal feed, paper and pulp industries [7-11]. Initial trials were performed in our laboratory showed that *A. terreus* is an excellent producer of xylanase when compared with other isolated fungi. Elephant foot yam (EFY), *Amorphophallus paeoniifolius* (Araceae), commonly known as Suran, is extensively grown in India as a cash crop due to its high industrial potential and gainful economic returns [12]. It is a good source of protein and carbohydrate, and is used as a vegetable [13]. EFY peels were sun-dried and used in present study as solid substrate for production under solid state fermentation of xylanase from *Aspergillus terreus* Thom which was isolated from elephant dung obtained from Indore Zoo [14,15]. The xylanase produced was partially purified using chromatographic techniques and characterized for its stability. kinetic parameters and effect of metal ions and detergents. This isolated xylanase from endophytic mesophilic fungus revealed notable thermostability and alkalinity which augments its biotechnological potential.

2. MATERIALS AND METHODS

2.1 Material

Birchwood xylan, DEAE–cellulose, Sephadex G-25, xylose and xylo-oligosaccharides were purchased from Sigma-Aldrich, USA. All the other chemicals used were of analytical grade and purchased locally. Agricultural residues collected from local fruit and vegetable shops were dried and used.

2.2 Inoculum Preparation and Fermentation Conditions

The inoculum was prepared by harvesting spores from a 5-day old fungal culture on PDA slants using sterile distilled water and filtered through

sterile glass wool. Spore suspension of 1×10^6 spores/ml, counted using Neubauer's counting chamber was used for inoculation. A 10 g sun dried substrate (2 mm particle size) was taken in 250 ml capacity Erlenmeyer flask, moistened with sterile distilled water to 70% (v/w) and autoclaved at 121^{o}C (15 lbs pressure) for 20 min. The SSF flasks were inoculated with 1 ml of inoculum (1×10^6 spores/ml) and incubated at 30^{o}C for 96 h.

2.3 Enzyme Extraction

The crude enzyme was extracted by adding 5 ml of 50 mM citrate buffer (pH 5± 0.5) per gram of fermented substrate and agitated at 120 rpm for 45 min at room temperature. The solid material was separated by passing the slurry through a muslin cloth. The filtrate was centrifuged at 1500 x *g* for 20 min at 0 to 4^{o}C temperature to obtain a clear supernatant and was used for xylanase assay.

2.4 Xylanase Enzyme Assay and Protein Estimation

Xylanase enzyme was assayed using birchwood xylan as a substrate. The 0.1 ml of enzyme solution was added to 0.9 ml of 0.2% birchwood xylan solution prepared in 0.05 M sodium citrate buffer, pH 5.5. The reaction mixture was incubated at 50°C for 10 min., and the The 3′ 5′-dinitrosalicylic acid (DNS) method (Miller, 1956; Bailey, 1992) was employed for determination used to determine of the released reducing sugar. One unit of endo-1, 4- β-xylanase was defined as the amount of the enzyme essential to liberate 1 µmole of xylose equivalent in one min under the conditions of enzyme assay [16]. The amount of the protein was determined by Lowry's method using bovine serum albumin (BSA) as standard (Lowry et al., 1951).

2.5 Purification of Xylanase

Xylanase was purified by employing ammonium sulphate precipitation, DEAE-cellulose ion exchange and Sephadex G-200 gel filtration chromatography. The crude enzyme extract obtained was subjected to ammonium sulphate precipitation at 0–90% saturation and placed for overnight incubation at 0–4^{0}C for complete precipitation of proteins. The protein precipitate was collected by centrifugation at 9000×*g* for 15 min and was suspended in 50 mM sodium phosphate buffer, pH 8.0. Desalting was performed using Sephadex G-25 column chromatography using the same buffer. 5 ml fractions were collected at a flow rate of 1.0 ml/min. 8 fractions of 5 ml each showing xylanase activity were pooled and applied on to DEAE-cellulose column (bed volume, 90 ml) previously equilibrated with 50 mM sodium phosphate buffer, pH 8.0. Unbound fractions were collected at a flow-rate of 1.0 ml/min by eluting with the same buffer. Bound proteins were eluted with a linear 0–1 M NaCl gradient, prepared in equilibrated buffer. The eluted fractions were checked for xylanase activity and absorbance was measured at 280 nm for the protein peaks. The xylanase fractions obtained after DEAE cellulose chromatography (fractions with highest specific activity) were pooled concentrated using reverse dialysis against solid sucrose for 3 h. The concentrated sample (around 1 ml) was loaded onto Sephadex G-200 (bed volume 70 ml) column and eluted with 50 mM sodium phosphate buffer (pH 8.0). The column was pre-equilibrated with the same buffer.

2.6 Native and SDS PAGE

Polyacrylamide gel electrophoresis was performed to determine purity and functionality using 10% resolving gel along with 0.1% xylan. After electrophoresis gel was The gel obtained after electrophoresis run was separated from glass plates and cut vertically into two halves, one was stained with Coomassie brilliant blue R-250 while another half was used for activity staining using 0.1% Congo red. For SDS PAGE, 10% separating gel with pH 8.8 and 4 % stacking gel with pH 6.8 were used. Resolved protein bands were visualized after staining with 0.1% (w/v) Coomassie brilliant blue R-250 dissolved in methanol, acetic acid, and distilled water (4:1:5; v/v/v).

2.7 Kinetic Parameters

The enzyme assay was carried out at different substrate concentrations ranging from 0.2- 30 mg/ml. The enzyme concentration was kept constant for all assays. Determination of *Km* and *Vmax* values was done by Lineweaver Burk plot.

2.8 Optimum Temperature and Thermal Stability

The optimum temperature was determined by assaying the enzyme activity at various temperatures, ranging from 10°C to 90°C for 15 min in 50 mM sodium phosphate buffer (pH 8.0)

under the standard xylanase assay conditions. For thermal stability, partially purified xylanase was incubated at different temperatures (40 to 90°C) and activity was checked after every 30 minutes for 3 h. The reaction tubes were covered by marbles, to prevent evaporation of the reaction mixture during the incubation at high temperatures. After cooling to room temperature, the xylanase activity was measured according to the standard assay conditions.

2.9 Optimum pH and pH Stability

The optimum pH of the enzyme was determined at different pH ranges from 3.0 to 12.0. The buffers used were as follows: 0.1 M sodium citrate (pH 3.0-5.0), 0.05 M sodium phosphate (pH6.0-8.0), 0.05 M Tris-HCl (pH 9.0-10.0), and 0.05 M glycine-NaOH (pH 11.0-12.0). The pH stability of partially purified xylanase enzyme was done after incubating enzyme xylanase in the different buffers described above, at 37°C for 3 h and followed by measuring the enzyme activity under the standard assay conditions.

2.10 Effect of Metal ions

The effect of metal ions on xylanase activity was examined using different ions such as Cu^{2+}, Co^{2+}, Fe^{2+}, Mn^{2+}, Ca^{2+}, Na^{+}, Mg^{2+}, Zn^{2+}, K^{+}, NH_4^{+} and Hg^{+} at 5- and 10-mM concentration. Xylanase activity was estimated after pre-incubating the enzyme with respective ions at 37°C for 1 h under standard assay conditions. Activity of the enzyme in the absence of any metal ion was considered as 100%.

3. RESULTS AND DISCUSSION

3.1 Purification of Xylanase

In the present study, xylanase purification from *Aspergillus terreus* EF1 was performed using ammonium sulfate precipitation, DEAE-cellulose and size exclusion chromatography. Specific activity of purified protein was found to be 111.59 U/mg with 14.6-folds purification and 39.48% yield. The molecular weight of the enzyme as determined using SDS PAGE was found to be nearly 87 kDa. A single band of protein was obtained exhibitis its monomeric nature. Lee et al. [17] purified thermostable xylanase from Basidiomycete, *Laetiporus sulphurous* employing three consecutive steps of purification and obtained a single protein band having molecular mass of 69.3 kDa achieving 9-folds purification and 68.7% yield. Knob and Carmona [18] reported two xylanases from *Penicillium sclerotiorum* using ion exchange and molecular exclusion chromatography. The estimated molecular masses of xylanases I and II by SDS PAGE were 23.9 and 33.1 kDa, respectively. Pal and Khanum (2011) purified an extracellular xylanase to homogeneity from *Aspergillus niger DFR-5* by using gel filtration chromatography over Sephadex G-100, and SDS -PAGE confirmed its monomeric nature. Hienen et al. (2014) used ion exchange chromatography to purify xylanase from *Fusarium heterosporum* and achieved 2.3-folds purity and reported 19.5 kDa molecular weight of xylanase. However, Seemakram et al. [19] reported mercury ion tolerant and low molecular weight of 25 kDa thermo-alkali stable xylanase from *Thermomyces dupontii KKU-CLD-E2-3,* which was purified to homogeneity using ammonium sulfate precipitation, Sephadex G–100 and DEAE cellulose resulting in 27.92-folds purification, specifc activity of 56.19 U/mg protein and a recovery yield of 2.01%.

3.2 Native PAGE and Activity Staining

Xylanase, purified using DEAE cellulose and Sephadex G-200 chromatography showed a single band on native gel polymerized with xylan. A single band of protein with xylanolytic activity was observed on the xylan gel, when stained by Congo red (Fig. 1).

The kinetic parameters *Km* and *Vmax* were determined by incubating the enzyme with different concentrations of birchwood xylan (0.2 to 30 mg/ml) under optimal conditions of enzyme assay. The values of *Km* and *Vmax* determined by Lineweaver Burk plot were found to be 0.016 mg/l and 102.64 U/mg protein, respectively (Fig.2). Kinetic studies help in understanding the affinity of xylanase for the substrate used. Zheng et al. (2013) reported *Km value* 0.548 mg/ml and *Vmax* 108.89 U/mg protein at 60^{o}C for the purified xylanase (Xyn II) from *Volvariella volvacea* using birchwood xylan. Sadaf and Khare [20] reported a *Km* value of 12.54 mg/ml and *Vmax* of 454 U/ml/min from *Sporotrichum thermophilic* using oat spelt xylan as substrate. The *Km* and *Vmax* of purified xylanase from *Aspergillus terreus UL4209* using oat spelt xylan were found to be 3.57mg/ml and 55.5 µmol/min/mg, respectively as reported by Chidi et al. [21]. Ribeiro et al. [22] reported *Km* and *Vmax of* 4.6 ± 0.3mg/ml and 82 µmol/min/mg, respectively, for xylanase from *Malbranchea pulchella* using birchwood xylan.

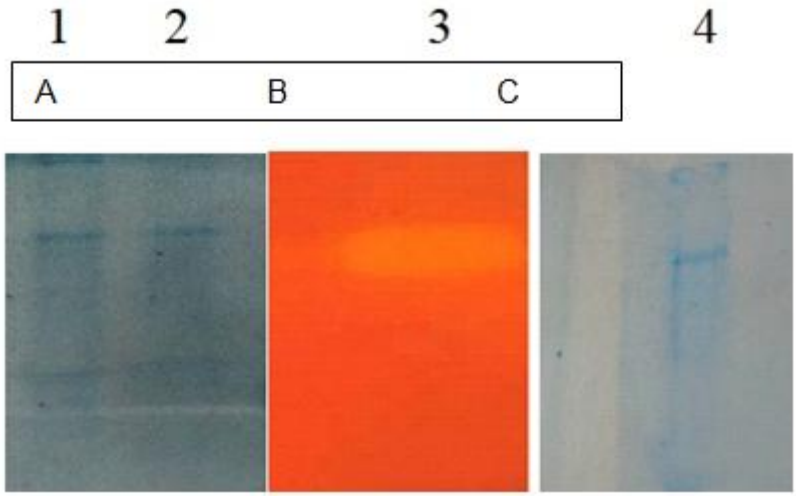

Fig. 1. Native PAGE study of partially purified xylanase enzyme: Fig 1 (A) Lane 1- showing DEAE cellulose purified sample, Lane 2- showing G-200purified enzyme, Fig 1 (B) 3- Activity Staining and Fig 1 (c) 4 - purified xylanase on 10% SDS PAGE

Table 1. Summary of purification steps of an extracellular xylanase from *A. terreus*

Purification step	Total activity (U)	Total protein (mg)	Specific activity (U/mg protein)	Purification fold	Yield (%)
Crude Enzyme	1950	255	7.64	1	100
Ammonium sulphate fraction (0-90%)	1830	146	12.53	1.64	93.84
Anion exchange chromatography on DEAE-cellulose	1329	31.6	37.33	4.80	68.15
Sephadex- G 200 Gel filtration chromatography	770	6.9	111.59	14.60	39.48

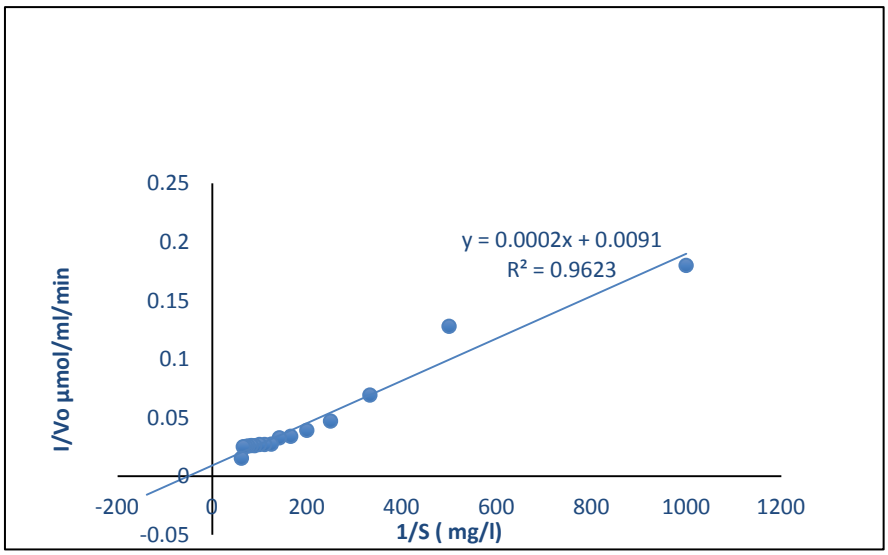

Fig. 2. Lineweaver Burk Plot for purified xylanase of *Aspergillus terreus* produced under SSF

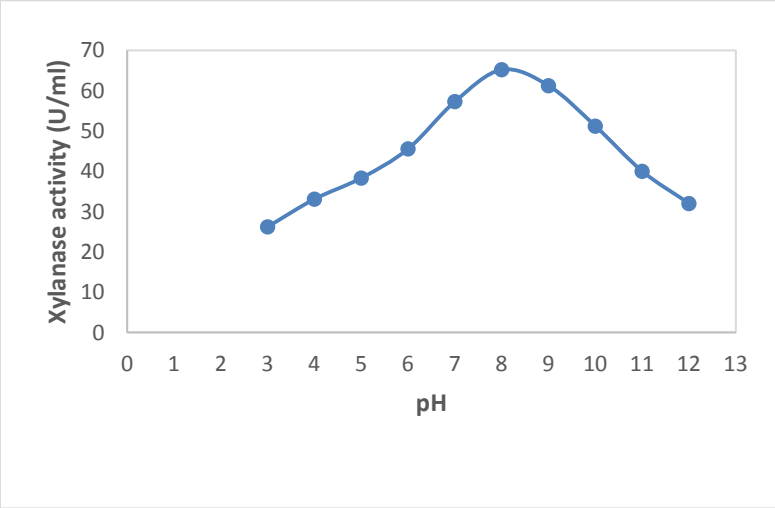

Fig. 3. Effect of different pH on xylanase activity

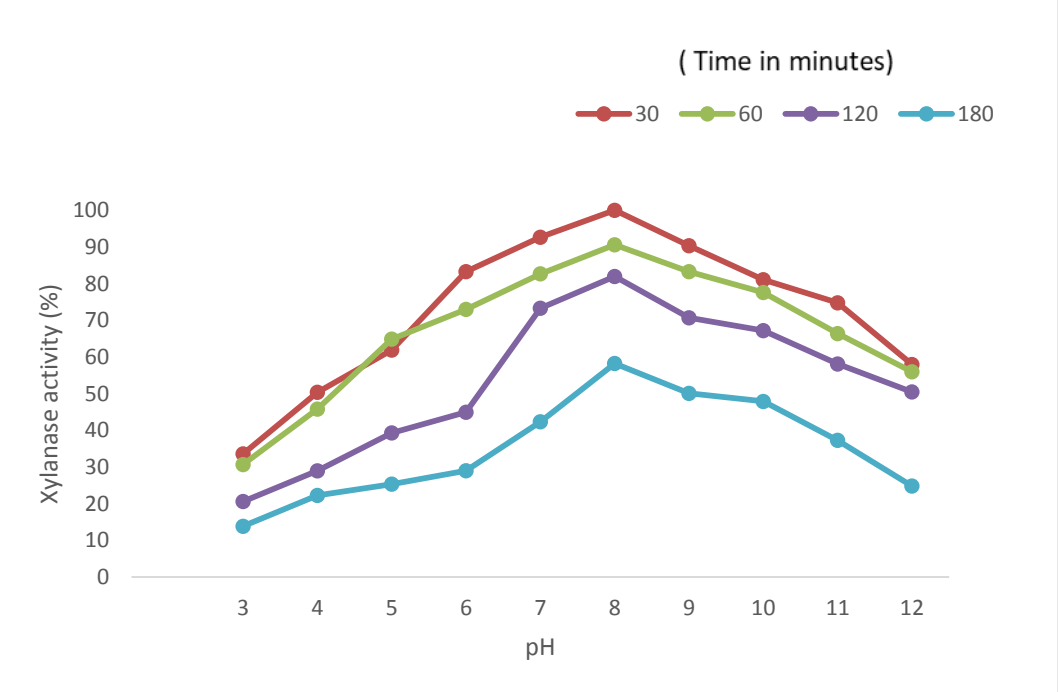

Fig. 4. Effect of pH on the stability of xylanase enzyme at different time intervals

3.3 Effect of pH on Xylanase Activity and pH Stability

The pH optima of xylanase enzyme from *Aspergillus terreus* Thom was found to be 8.0 (Fig. 3). Several researchers have shown the pH optima of xylanase between 4.5 [23] to pH 9 [19]. Bajaj et al. [24] also reported optimum pH 8 for xylanase activity of an alkali tolerant fungus from *A. fumigatus* MA28. Gomes et al., [23] reported wide range of pH wide range of pH stability and tolerance to ethanol, xylose and glucose for xylanase from *A. fumigatus*. Seemakram et al. [19] reported thermo-alkali stable and mercury ion tolerant xylanase from *Thermomyces dupontii* KKU-CLC-E23.

The A. terreus xylanase showed considerable stability at pH 6, 7, 8, 9, 10 and 11 with 83.28%, 92.66%,100%, 90.35%, 81.08%, and 74.78%, respectively. After 1 h, xylanase retained 91% activity at pH 8 and more than 75 % activity at pH 9 and 10. After 2.5 h of incubation, enzyme

retained more than 60% activity at pH 8-10 and nearly 30% activity at pH 6 and 7 (Fig.4). In 1992, Mathrani and Ahring reported that thermophilic and alkaliphilic xylanase from *Dictyoglomus* isolates retained 100% activity at pH range 5.5–9.0. An alkali-tolerant xylanase from *Aspergillus fischeri* was reported to exhibit remarkable stability at pH 9.0 [25]. Chipeta et al. [26] reported xylanolytic activity from A. *oryzae* to remain stable at 50^{o}C over pH range of 4.5-10. Rhomdane et al. [27] reported the maximum xylanase activity at 75^{o}C from *Talaromyces thermophilus* at pH 8 which exhibited a notable stability and retained 100% of its original activity at 50°C for 7 days at pH 7.0–8.0. The half-lives of the enzyme were 4 h at 80°C, 2 h at 90°C, and 1 h at 100°C. Kocabas et al. (2015) reported xylanase from *Scytalidium thermophilium* ATCC No. 16454 which retained 80% of its maximum activity at pH 8.0. It was also reported that enzyme exhibited most stability in the pH range of 6-8 after 6 h of incubation and highest pH stability at pH 8. Seemakram et al. [19] reported that the xylanase can maintain more than 70% of the original activity in pH range of 7-10 after incubating at 70°C for 1.5 h in its purified state. The xylanase enzyme from *Aspergillus terreus* Thom. has the potential to be used for its alkaline characteristic and stability over a broad pH range and at high temperature.

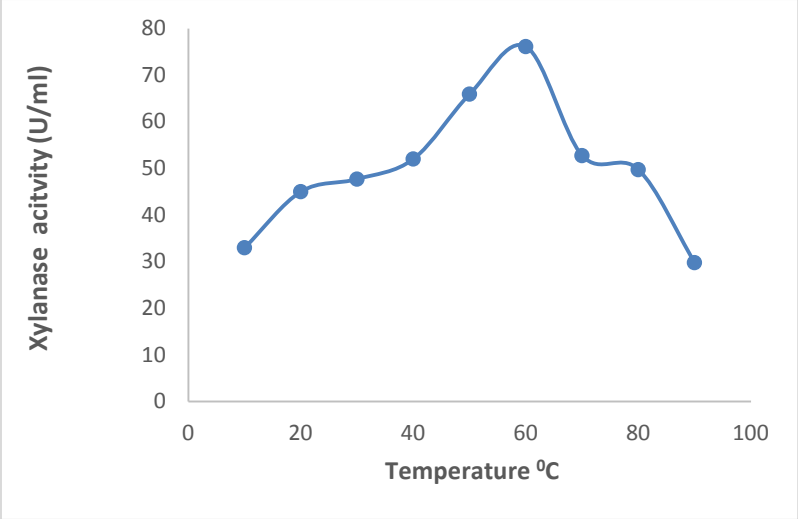

Fig. 5. Effect of Temperature on xylanase enzyme activity

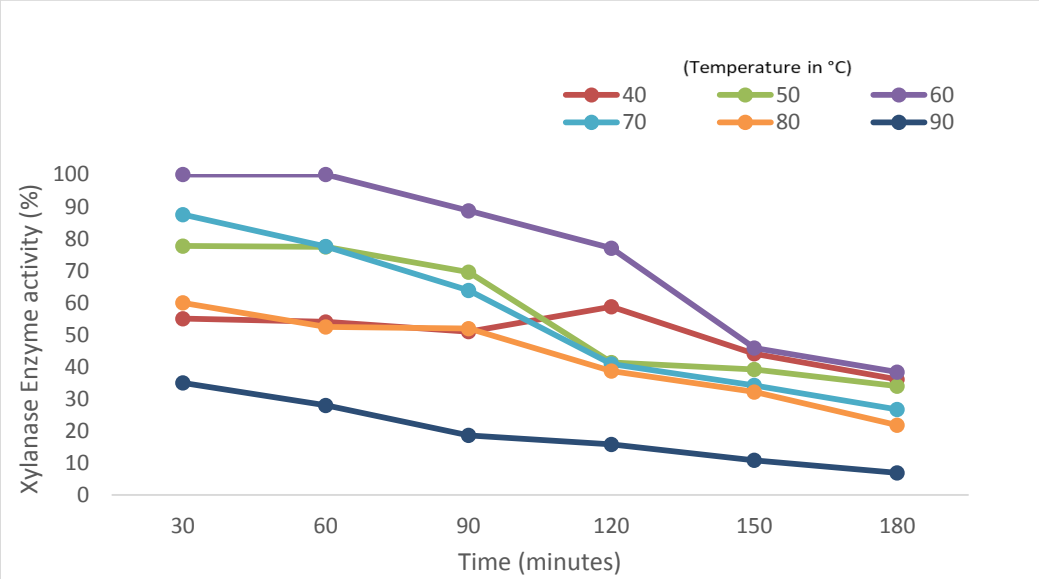

Fig. 6. Effect of temperature on thermostability of xylanase enzyme

3.4 Effect of Temperature on Xylanase Activity and Stability

The optimum xylanase activity 76.1±5.5 U/ml was found at 60˚C (Fig. 5). Previous reports revealed that xylanase isolated from members of the genus *Aspergillus* showed maximal activity at 50°C [28,29]. In 2005, Kolenova et al. purified Xyn B and Xyn C from *Schizophyllum commune*, which were most active at 50°C. Most of the xylanolytic enzymes showed maximum activity at 50-60^{0}C [30,31,32]. Bakri et al. [33] revealed that xylanases from *A. tubingensis* and *A. terreus* exhibited optimum activity at 65°C. Similarly, xylanase has been reported from *T. thermophilus* with temperature optima of 75–80°C [34]. However, Chidi et al. [21] reported xylanase activity from *Aspergillus terreus* UL4209 strain at 50°C and enzyme retained 95% activity at 35-40°C after 4 h of incubation at pH 6 and at 50°C the half-life was 5.8 h. Bajaj et al. [24] reported optimum temperature 50°C for xylanase activity and temperature optima of 80°C for xylanase from *T. dupontii* KKU-CLC-E23.

Thermal stability studies showed that the *A. terreus* xylanase retained 100% of the enzyme activity at 60^0 C for 60 min. At 50^0 C and 70^0 C, xylanase retained 69.54% and 63.83% activity respectively, after 1.5 h of incubation (Fig.6). At temperature 80^0 C and 90^0 C, the enzyme retained 59.98% and 35% activity, respectively after 30 min incubation. An extensive range of thermostability has been reported for xylanases from mesophilic fungi and thermophilic fungi [35]. Kitpreechavanich et al. [36] reported xylanase stability at 65°C, produced by *Aspergillus fumigatus*. A strain of *Aspergillus niger* reported by John et al. [37] which produced two enzymes with extensive temperature stability between 65°C and 80°C. Gawande and Kamat (1998) reported a half-life of xylanase purified from two *Aspergillus* sp. at 50^{0}C for 240 and 260 min and stable only for 10 min at 50^{o}C and 35^{o}C, respectively. Kocabas et al. (2015) reported xylanase from *Scytalidium thermophilium* ATCC No. 16454 which retained more than 95% activity at 40^{o}C after 4 h of incubation. He also reported that purified xylanase retained 98%, 85% and 50% of its initial activity after 1h incubation at 40, 50 and 60^{o}C and after 2 h of incubation at 70^{o}C enzyme activity was entirely lost. Atalla et al. (2020) reported crude xylanase from *Chaetomium globosum* which on increasing incubation time from 30 min at 40°C decreased xylanase activity upto 45% while at 60°C enzyme lost 85% of its activity. Thus, above mentioned reports prove the ability of xylanase to work under broad temperature ranges and capable to tolerate high temperature processes in multiple industries.

3.5 Effect of Metal Ions on Xylanase Activity

The effect of various metal ions on xylanase enzyme activity is shown in Table 2. The data indicated that the metal ions such as Zn^{2+}, Ca^{2+}, Fe^{3+}, Mg^{2+} and NH_4^+ when added at 10 mM concentration, stimulated the activity by 187%, 129%, 118%, 123% and 146%, respectively. These metal ions were used as salts of sulphate. However, the activity was mildly inhibited by EDTA at 10 mM concentration. The Cu^{2+} and Hg^{2+} completely inhibited xylanase activity at 10 mM concentration. Hg^{2+} inhibition of xylanase has been associated with the presence of tryptophan residues which oxidize indole ring, thereby inhibiting the enzyme activity. Few reports occur on the action of ionic detergents such as SDS in xylanase, at low concentration (1-2mM) it can stimulate enzyme activity whereas in higher concentration (10-20 mM), they can cause inhibitory effect [38]. Similarly, this study can be considered for metal ions and non-ionic detergents exhibiting this property. In the present study also, surfactants like Tween 20 and Triton X increased the activity at 5 mM concentration while decline in activity occurs at higher concentration of 10 mM. Urea enhanced xylanase activity at both 5 mM and 10 mM concentrations upto 113% and 139%, respectively [39-41]. However, 5 mM EDTA increased activity upto 10% and at its higher concentration, enzyme activity decreased. The interaction between cations and amino acid residues involved in enzymatic catalytic domains modulates enzyme activity either positively or negatively [42-45]. In the present study, Zn^{2+} ions outstandingly stimulated xylanase activity showing its involvement in structural properties of enzyme. Apart from Zn^{2+}, Mn^{2+}, Ca^{2+}, Fe^{3+}, Mg^{2+} and NH_4^+ ions were also found to stimulate xylanase activity signifying the possible role of both metal ions as cofactors for enzyme-substrate reactions, and provide a stability effect on many other enzymes [46]. Roy et al. [47] revealed the strong inhibition of xylanase activity from *Simplicillium obclavatum* MTCC9604 by addition of 3 mM and 6 mM of Mg^{2+}, Ni^2, Cu^{2+} and Hg^{2+} whereas 3 mM concentration of Zn^{2+}enhanced the xylanase activity.

Table 2. Effect of metal ions and solvents on the relative activity of a purified xylanase from *A. terreus*

Metal ions and Solvents	5mM concentration Enzyme activity (%) [a]	10mM concentration Enzyme activity (%) [a]
Control	100 ± 1.12	100 ± 1.12
$CuSO_4$	5.13 ± 2.12	6.45 ± 2.14
$CoCl_2$	95.40 ± 1.02	48.75 ± 2.08
$HgCl_2$	0.0	0.0
$MnSO_4$	112.14 ± 1.25	128 ± 2.12
$ZnSO_4$	107 ± 2.10	187 ± 1.62
$FeSO_4$	128 ± 1.29	118 ± 1.72
$CaCO_3$	109 ± 1.38	129 ± 1.10
$MgSO_4$	115 ± 2.52	123 ± 2.19
$(NH_4)_2SO_4$	134 ± 1.62	146 ± 2.06
Tween 20	131 ± 1.92	85 ± 1.43
Tween 80	112 ± 2.2	78.65 ± 1.54
EDTA	10 6± 2.70	81.81 ± 1.32
Urea	113 ± 1.16	139 ± 2.59
Triton- X-100	123 ± 1.15	118 ± 1.81

[a] *Values are means ±SD of three experiments*

4. CONCLUSION

The xylanase enzyme isolated from *Aspergillus terreus* Thom was produced employing SSF using EFY peels as solid substrate. Xylanase was purified and characterized by chromatographic techniques and was found to be utilizing a biowaste Elephant Foot Yam peel. The results obtained in the present study showed that optimum pH and temperature of xylanase was 8 and 60 °C. Thus, xylanase isolated here was found to be active over a pH range of 6-11 and temperature range of 40°C to 80°C, indicating its alkali tolerant and thermostable characteristics which is the major requirement of several industries. Zn^{2+} ions enhanced xylanase activity by 187% followed by Ca^{2+} (129%), Mg^{2+} (123%), NH_4^+ (146%) and Fe^{3+} (118%) at 10 mM concentration. However, Cu^{2+} and Hg^{2+} completely inhibited xylanase activity. The present study reports A. *terreus* Thom xylanase which due to its stability and alkalinity can be applied for utilization individually or in consortia for various industries including processing of pulp fibers, bread baking, bioethanol and hydrolysis of complex sugars in agricultural, industrial and municipal wastes and for other bioconversions.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

ACKNOWLEDGEMENTS

The authors acknowledge the facilities present in the School of Biotechnology, Devi Ahilya University, Indore and also acknowledge the facility provided at the Maharaja Ranjit Singh College of Professional Sciences, Indore. M.P.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Pal A, Khanum F. Covalent immobilization of xylanase on glutaraldehyde activated alginate beads using response surface methodology: Characterization of immobilized enzyme. Process Biochem. 2011;46:1315–1322.
2. Verma D, Satyanarayana T. Molecular approaches for ameliorating microbial xylanases. Bioresour. Technol., 2012; 117:360–367. Available:https://doi.org/10.1016/j.biortech.2012.04.034
3. Bhardwaj N, Kumar B, Verma P. A detailed overview of xylanases: An emerging

biomolecule for current and future prospective. Bioresour. Bioprocess. 2019; 6(1):1-36.

4. Walia A, Guleria S, Mehta P, Chauhan A, Parkash J. Microbial xylanases and their industrial application in pulp and paper biobleaching: A review. 3 Biotech. 2017; *7*(1):11.
DOI: 10.1007/s13205-016-0584-6
5. Romero-Fernández M, Moreno-Perez S, Orrego AH, de Oliveira SM, Santamaría RI, Díaz M, Guisan JM, Rocha-Martin J. Designing continuous flow reaction of xylan hydrolysis for xylooligosaccharides production in packed-bed reactors using xylanase immobilized on methacrylic polymer-based supports. Bioresour. Technol. 2018;266:249-58.
6. Rana KL, Kour D, Sheikh I, Dhiman A, Yadav N, Yadav AN, Rastegari AA, Singh K, Saxena AK. Endophytic fungi: Biodiversity, ecological significance, and potential industrial applications. InRecent advancement in white biotechnology through fungi. Springer. 2019;1-62.
7. Beily P. Biochemical aspects of the production of microbial hemicellulase. In: Coughlan MP, Hazlewood GP (eds) Hemicellulose and hemicellulases. Portland Press.1993;29-51.
8. Chandra RK, Chandra TS. Production of cellulase-free xylanase from an alkalo-tolerant Aspergillus f*ischeri* Fxn1. Biotechnol. Lett. 1995;17:309–314.
9. Gawande PV, Kamat MY. Production of *Aspergillus* xylanase by lignocellulosic waste fermentation and its application. J. Appl. Microbiol.1999;87:511-519.
10. Heinen PR, Bauermeister A, Ribeiro LF, Messias JM, Almeida PZ, Moraes LA, Vargas-Rechia CG, De Oliveira AH, Ward RJ, Kadowaki MK, Jorge JA. GH11 xylanase from Aspergillus tamarii Kita: Purification by one-step chromatography and xylooligosaccharides hydrolysis monitored in real-time by mass spectrometry. International Journal of Biological Macromolecules. 2018;108:291-99.
11. Juturu V, Wu JC. Microbial xylanases: Engineering, production and industrial applications. Biotechnol adv. 2012; 30(6):1219-1227.
12. Jogi P, Lahre N. Impact of front-line demonstration on yield and economics of elephant foot yam (Amorphophallus *paeoniifolius*) in Mungeli District of Chhattisgarh. J. pharmacogn. phytochem. 2020;9(5):3205-3208.
13. Chattopadhyay A, Saha B, Pal S, Bhattacharya A, Sen H. Quantitative and qualitative aspects of elephant foot yam. Journal of Vegetable Science, 2009; 16(1):73-84.
14. Kocabas DS, Ozben N. Co-production of xylanase and xylooligosaccharides from lignocellulosic agricultural wastes. *RSC Advances*, 2014;4(50):26129-26139.
15. Kocabaş DS, Güder S, Özben N. Purification strategies and properties of a low-molecular weight xylanase and its application in agricultural waste biomass hydrolysis. J. Mol. Catal. B Enzym. 2015;115**:**66-75.
16. Bhalla A, Bischoff, KM, Sani RK. Highly thermostable xylanase production from a thermophilic *Geobacillus sp.* Strain WSUCF1 utilizing lignocellulosic biomass. Front. Bioeng. Biotechnol. 2015;3:84.
DOI: 10.3389/fbioe.2015.00084
17. Lee JW, Park JY, Kwon M, Choi IG. Purification and characterization of a thermostable xylanase from the brown-rot fungus *Laetiporus sulphureus.* J. Biosci. Bioeng. 2009;107(1):33-7.
18. Knob A, Carmona EC. Purification and characterization of two extracellular xylanases from *Penicillium sclerotiorum*: A novel acidophilic xylanase. Appl. Biochem. Biotechnol. 2010;162(2):429-43.
19. Seemakram W, Boonrung S, Kokaew U, Aimi T, Boonlue S. Optimization of culture conditions for xylanase production from cellulase-free xylanase-producing thermophilic fungus, *Thermomyces dupontii* KKU− CLD− E2− 3. Chiang Mai J. Sci. 2020;47:391-402.
20. Sadaf A, Khare SK. Production of Sporotrichum *thermophile* xylanase by solid state fermentation utilizing deoiled Jatropha curcas seed cake and its application in xylooligosachharide synthesis. Bioresour. Technol. 2014; 153:126-130.
Available:https://doi.org/10.1016/j.biortech.2013.11.058
21. Chidi SB, Godana, B, Ncube I, Van Rensburg EJ, Cronshaw A, Abotsi EK. Production, purification and characterization of celullase-free xylanase from *Aspergillus terreus* UL 4209. Afri J Biotechnol. 2008;*7*(21).
22. Ribeiro LF, De Lucas RC, Vitcosque GL, Ribeiro LF, Ward RJ, Rubio MV, Maria de

Lourdes TM. A novel thermostable xylanase GH10 from *Malbranchea pulchella* expressed in *Aspergillus nidulans* with potential applications in biotechnology. Biotechnology for biofuels. 2014;7(1):1-11.
Available:https://doi.org/10.1186/1754-6834-7-115

23. Gomes AF, dos Santos BS, Franciscon EG, Baffi MA. Substract and temperature effect on xylanase production by Aspergillus fumigatus using low -cost agricultural wastes. Bioscience Journal. 2016;32(4).
24. Bajaj BK, Sharma M, Sharma S. Alkalistable endo-β-1, 4-xylanase production from a newly isolated alkali tolerant Penicillium sp. SS1 using agro-residues. 3 Biotech. 2011;1(2):83-90.
25. Raj KC, Chandra TS. Microbiol. Lett. 1996;145:457–461.
26. Chipeta ZA, du Preez JC, Szakacs G, Christopher L. Xylanase production by fungal strains on spent sulphite liquor. Appl Microbiol Biotechnol. 2005;69:71–78
27. Romdhane IB, Achouri IM, Belghith H. Improvement of highly thermostable xylanases production by *Talaromyces thermophilus* for the agro-industrials residue hydrolysis. Appl. Biochem. Biotechnol. 2010;162(6):1635-1646.
28. Carmona EC, Fialho MB, Buchgnani ÉB, Coelho GD, Brocheto-Braga MR, Jorge JA. Production, purification and characterization of a minor form of xylanase from *Aspergillus versicolor*. Process Biochem. 2005;40(1):359-364.
29. Mendicuti Castro LP, Trejo-Aguilar BA, Aguilar Osorio G. Thermostable xylanases produced at 37^{o}C and 45^{o} C by a thermotolerant Aspergillus strain. FEMS Microbiol. Lett.1997;146(1):97-102.
30. Saha BC. Production, purification and properties of xylanase from a newly isolated Fusarium *proliferatum.* Process Biochem. 2002;37:1279-1284.
31. Ahmed S, Imdad SS, Jamil A. Comparative study for the kinetics of extracellular xylanases from *Trichoderma harzianum* and *Chaetomium thermophilum*. Electron J. Biotechnol. 2012;15(3):3-3.
DOI: 10.2225/vol15-issue3-fulltext-2
32. Kamble RD, Jadhav AR. Production, purification and characterisation of alkali stable xylanase from Cellulosimicrobium sp. MTCC 10645. Asian Pac. J. Trop. Biomed. 2012;1790-1797.
33. Bakri Y, Masson M, Thonart P. Isolation and identification of two new fungal strains for xylanase production. Applied biochemistry and biotechnology. 2010; 162(6):1626-34.
34. Maalej I, Belhaj I, Masmoudi NF, Belghith H. Highly thermostable xylanase of the thermophilic fungus *Talaromyces thermophilus*: Purification and characterization. Appl. Biochem. Biotechnol. 2009;158(1):200-212.
Available:https://doi.org/10.1007/s12010-008-8317-x
35. Chadha BS, Kaur B, Basotra N, Tsang A, Pandey A. Thermostable xylanases from thermophilic fungi and bacteria: current perspective. Bioresour. Technol. 2019;277:195–203.
Available:https://doi.org/10.1016/j.biortech.2019.01.044
36. Kitpreechavanich V, Hayashi M, Nagai S. Production of xylan-degrading enzymes by thermophilic fungi, *Aspergillus fumigatus* and *Humicola lanuginosa.* J. Ferment. Technol. 1984;62(1):63-69.
37. John M, Schmidt B, Schmidt J. Purification and some properties of five endo-1, 4-β-D-xylanases and β-D-xylosidase produced by a strain of *Aspergillus niger*. Can. J. Biochem. 1979;57(2):125-134.
38. Debeche T, Cummings N, Connerton I, Debeire P, O'Donohue MJ. Genetic and biochemical characterization of a highly thermostable alpha-L-arabinofuranosidase from *Thermobacillus xylanilyticus* Appl. Environ. Microbiol. 2000;66:1734-1736.
39. Miller GL. Use of dinitrosalicylic acid reagent for determination of reducing sugar. Ana. Chem. 1959;31:426-428.
40. Raj A, Kumar S, Singh SK. A highly thermostable xylanase from *Stenotrophomonas maltophilia*: Purification and partial characterization. 2013;1-8.
Available:http://dx.doi.org/10.1155/2013/429305
41. Zheng HC, Sun MZ, Meng LC, Pei HS, Zhang XQ, Yan Z, Zeng WH, Zhang JS, Hu JR, Lu FP, Sun JS. Purification and characterization of a thermostable xylanase from Paenibacillus sp. NF1 and its application in xylooligosaccharides production. J. Microbiol. Biotechnol. 2014; 24(4):489-96.

42. Kolenová K, Vršanská M, Biely P. Mode of action of endo-β-1, 4-xylanases of families 10 and 11 on acidic xylooligosaccharides. J of biotechnol. 2006;121(3):338-45.
43. Li L, Tian H, Cheng Y, Jiang Z, Yang S. Purifcation and characterization of a thermostable cellulase-free xylanase from the newly isolated *Paecilomyces themophila*. Enzyme Microb. Technol., 2006;38:780–787.
44. Lineweaver H, Burk D. The determination of enzyme dissociation constants. Journal of the American chemical society. 1934;56(3):658-666.
45. Mathrani IM, Ahring BK. Appl. Microb. Biotechnol. 1992;38:23–27.
46. Sorgatto M, Guimarães NC, Zanoelo FF, Marques MR, Peixoto-Nogueira SC, Giannesi GG. Purification and characterization of an extracellular xylanase produced by the endophytic fungus, Aspergillus terreus, grown in submerged fermentation. African Journal of Biotechnology. 2012;11(32):8076-84.
47. Roy S, Dutta T, Sarkar TS, Ghosh S. Novel xylanases from *Simplicillium obclavatum MTCC 9604*: comparative analysis of production, purification and characterization of enzyme from submerged and solid-state fermentation. Springerplus. 2013;2(1):1-10. Available:https://doi.org/10.1186/2193-1801-2-382

Peer-review history:
The peer review history for this paper can be accessed here:
https://www.sdiarticle5.com/review-history/81968