Submerged Fermentation, Partial Purification and Characterization of Xylanase Isolated from *Fusarium Solani* for Biotechnological Applications

ABSTRACT

Xylanase enzymes play a vital role in the breakdown of hemicellulose biomass and have diverse industrial applications. This study focused on the production, partial purification, and characterization of xylanase from *Fusariun solani* isolated from the gut of longhorn beetles (*Cerambycidae latreille*). Xylanase was produced by submerged fermentation using carboxymethyl cellulose as the carbon source. The crude enzyme extract showed xylanase activity of 0.095 U/mL. Partial purification of xylanase was achieved through ammonium sulfate precipitation (60 % saturation) and ion exchange chromatography using DEAE sephacel resin, resulting in 42.06 % yield and 3.63-fold purification. The purified xylanase exhibited optimal activity at an alkaline pH of 4.0 and retained stability over a broad pH range from 5.0 to 12.0. It showed optimum activity at 40 °C, displaying thermostability up to 60°C for 3 hours. Metal ions including Al^{2+}, Ca^{2+}, and Hg^{2+} stimulated cellulase activity while Mg^{2+} inhibited it. Kinetic analysis revealed a Km of 0.3741 mM and a Vmax of 0.5363 mg/ml/min. This study demonstrates the potential biotechnological application of xylanase derived from insect gut microbes, for use in biomass hydrolysis, food processing, feed processing and other industrial processes requiring hydrolytic enzymes stable under alkaline conditions and moderate heat.

KEYWORDS: Submerged fermentation, enzyme activity, physicochemical properties, inhibitors, bioprocess.

1.0 INTRODUCTION

Xylanase, an enzyme crucial for the degradation of xylan, a major component of plant cell walls. It plays a critical role in several industrial processes including paper and pulp production, animal feed formulation, and biofuel generation (Jacomini et al., 2023; Almeida et al., 2021; Collins et al., 2005). Xylanases are a group of enzymes that belong to the glycoside hydrolase family. They catalyze the hydrolysis of xylan, a hemicellulose component found in plant cell walls, into xylose and other xylo-oligosaccharides. The structure of xylanase typically includes a catalytic domain that is responsible for the enzymatic activity and, in some instances, one or more carbohydrate-binding modules (CBMs) that enhance substrate binding (Agrawal et al., 2022; Abishna, et al., 2020).

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Carbohydrate-binding modules (CBMs) in xylanases enhance enzyme efficiency by facilitating binding to insoluble xylan substrates. These modules localize the enzyme on the substrate surface, increasing its effective concentration at the site of action and improving hydrolysis efficiency. CBMs are categorized into families based on carbohydrate-binding specificity and structural characteristics (Agrawal et al., 2022; Srivastava et al., 2022). Their ability to target and degrade complex, insoluble xylan substrates, such as those found in plant cell walls, highlights their importance in biotechnological applications. By optimizing enzyme-substrate interactions, CBMs enable more effective utilization of xylanases in industrial processes and environmental applications aimed at biomass conversion and biofuel production (Bharti, et al., 2023; Kumar et al., 2023; Zhang et al., 2023).

The growing demand for xylanase has driven extensive research efforts focused on enhancing its production and optimizing its efficiency. Among potential producers, *F. solani*, a fungal species recognized for its pathogenic impact on plants, has emerged as particularly promising due to its ability to produce substantial quantities of this enzyme (Khare et al., 2018). The optimization of xylanase production from F. solani involves a comprehensive exploration of the underlying mechanisms governing its synthesis. Understanding these mechanisms is essential for identifying and mitigating factors that can influence enzyme yield. Factors such as culture conditions, substrate availability, pH, temperature, and oxygen levels all play critical roles in determining the productivity of xylanase-producing microorganisms (Adhyaru et al., 2017). Efforts are also directed towards genetic and metabolic engineering approaches to enhance xylanase production in *F. solani*. These strategies aim to modify the genetic makeup or metabolic pathways of the fungus to increase enzyme expression levels and improve overall production efficiency. Additionally, Bioprocess engineering optimizes fermentation conditions and kinetics, enhancing xylanase production yields (Fang et al., 2022). Enzymes have proven to be beneficial in industrial applications such as bioconversion of lignocellulosic biomass to value-added products like biofuels and biochemicals (Mehmood et al., 2019). *F. solani*, being a fungal species, offers advantages such as robust growth on inexpensive substrates and potentially high xylanase production yields, making it economically viable for enzyme production at scale (Bharti et al., 2023). Extracting xylanase from *F. solani* offers advantages in enzyme properties such as thermostability, pH tolerance, and broad substrate specificity, crucial for industrial processes under harsh conditions (Khalil et al., 2022). Additionally, studying xylanases from this fungal source contributes to biodiversity research, providing insights into fungal enzyme evolution and adaptation strategies (Ong et al., 2022). Understanding the biochemical and kinetic characteristics of *F. solani* xylanase supports its optimization for biotechnological applications, enhancing enzymatic hydrolysis of complex polysaccharides (Bhardwaj et al., 2019). Therefore, the aim of this study was to investigate the potential of the filamentous fungus *F. solani* as a novel and efficient source of xylanase enzyme for various biotechnological applications by conducting detailed studies on the production, partial purification, and characterization of its xylanase This research not only advances knowledge of fungal xylanases but also enhances sustainable bioprocessing technologies, promising both scientific insights and practical industrial benefits (Ezeilo et al., 2020).

2.0 MATERIALS AND METHOS

2.1 Materials

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2.1.1 Source of Organism

Xynalase which had been previously isolated from *F. solani* was collected at Enzyme Biotechnology Unit laboratory from the Department of Biochemistry, Federal University of Technology Akure, Nigeria.

2.1.2 Reagents/ Chemicals

Chemical reagents such as, Xylan (substrate), dinitrosalicylic acid (DNSA), diethylaminoethyl (DEAE), US while chemicals such as agar, sodium acetate, citric acid, sulphuric acid, distilled water, hydrochloric acid (HCl), sodium hydroxide, Tris-HCl buffer, sodium chloride, magnesium sulphate, glycine, ethylenediaminetetraacetic acid (EDTA), s odium tartarate, copper sulfate, calcium chloride, manganese sulphate, monopotassium sulphate, potassium chloride, iron chloride, sodium hydroxide, dithiothretol, β-mercaptoethanol, sodium dodecyl sulfate, L-cysteine, tween 20, Triton X-100, ethanol, isopropanol, peptone, barium chloride, ammonium sulfate.

2.1.3 Equipment

The equipment used for this research were; portable pressure steam sterilizer (Model YX-18LM), UV–visible spectrophotometer (Axiom 721 vis spectrophotometer UK), pH meter (Philips India), water bath shaker (WHY-2, USA), water bath (HH-W420 thermostatic water cabinet, UK), refrigerated centrifuge (AFI, India), analytical and top loading balance (OHAUS Corporation, US). Other materials include glassware, spatula, glass stirrer, inoculating loop and microliter pipette.

2.2 Methods

2.2.1 Preparation and Seed culture

Potato dextrose agar (PDA) of 3.9 g was dissolved in 100ml of distilled water, and was sieved to remove agar and thereafter sterilized using autoclave at 121 °c for 15 min after which it was allowed to cool before being inoculated with *F. solani* followed by incubation with water bath shaker at 30 °c, 150 rpm for 48hrs.

2.2.2 Production of Xylanase isolated from *Fusarium solani*

twenty grams xylan, 7g of peptone, 0.005g of $CaCl_2$, 0.23g of KH_2PO_4, and 0.05g of $MgSO_4$, 0.002g of $ZnSO4$, 0.009g of FeSO4, 0.23g of KCl, were measured and dissolved in 1000mL of distilled water in a conical flask. The solution was thoroughly mixed. The resulting mixture was divided among four conical flasks, autoclaved at 121 °C for 15 minutes, and then allowed to cool. Afterward, 3 milliliters of the seed culture were inoculated into each flask. The flasks were placed on an orbital shaker at 150 rpm and incubated at 30 °C for 36-48 hours. Following incubation, the culture fluid was centrifuged at 40,000 rpm

for 15 minutes using a refrigerated centrifuge. The supernatant, considered the crude enzyme, was collected and stored at 4 ℃. Additionally, the volume of the crude enzyme was measured by weight.

2.2.3 Determination of Xylanase Activity

Xylanase activity was determined by mixing 0.9 ml of 1 % (w/v) birch wood xylan (prepared in 50 mM Na-citrate buffer, pH 5.3) with 100 µL of aliquot enzyme. The mixture was incubated at 50 ºC for 5 min and the reaction was stopped by adding 1000 µL of 3,5-dinitrosalicylic acid (DNSA). The test tubes containing the reaction mixture were boiled for 5 min and then cooled at room temperature. After cooling, the absorbance of the colour developed was read at 540 nm. The blank was set up without the enzyme and treated in the same condition as sample.

2.2.4 Determination of Protein Concentration

The protein concentration was routinely determined according to the method of Bradford (1976) using Bovine serum albumin standard and the absorbance was read against blank at 595 mM with a spectrophotometer.

2.2.5 Purification of Xylanase

2.2.5.1 Ammonium sulfate Precipitation

400 mL of the crude enzyme was prepared and brought to 60 % saturation with solid ammonium sulfate at 4 ℃. The quantity, in grams, equivalent to 60 % saturation of solid ammonium sulfate was determined using a standard calculator available at www.encorbio.com. The solid ammonium sulfate was gradually added in small amounts while gently stirring to prevent ladder (foaming) and left to settle overnight. The resulting precipitate was centrifuged at 10,000 rpm for 10 minutes, and the pellet was then diluted using 0.02 M citrate buffer (pH 5.3) and stored in the refrigerator.

2.2.5.2 Dialysis of Crude Enzyme

The pellet obtained was dialyzed against 0.02 M citrate buffer (pH 5.3) at 4 ºC using a pre-treated dialysis bag, with three buffer changes. Subsequently, the partially purified sample was assayed for both enzyme activity and protein content.

2.2.5.3 Ion Exchange Chromatography

The enzyme obtained from dialysis was added to a well-packed DEAE Sephadex column sized at 2.5 cm × 1.25 cm. The resin was equilibrated with 0.02 M citrate buffer at pH 5.3, and the enzyme was introduced into the column. Unbound proteins were collected initially, followed by the elution of bound proteins using 11.6g NaCl dissolved in the buffer solution. Protein presence was confirmed by measuring the collected fractions at 280 nm, while enzyme activity was assayed following the standard assay procedure. Fractions with xylanase activity were pooled together and stored at 4 ℃.

2.2.6 Characterization of Partially Purified Xylanase

2.2.6.1 Effect of pH on the Activity of Partially Purified Xylanase

The effect of pH on the activity of xylanase was investigated by carrying out xylanase assay of various pH ranging from 2.0 to 12.0. The buffering system consisted 50 mM glycine-HCl (pH 2.0 and 3.0), citrate buffer (pH 4.0 and 5.0), K_2HPO_4/ KH_2PO_4 buffer (pH 6.0 and 7.0), Tris-HCl (pH 8.0, 9.0 and 10.0) and Tris-N_aOH buffer (pH 11.0 and 12.0) were employed. The enzyme assay of purified xylanase was carried out according to the standard assay procedure.

2.2.6.2 Effect of Temperature on the Activity of Partially Purified Enzyme

The effect of temperature on the purified enzyme was determined by varying the temperature condition of the reacting mixture. The reacting mixture was incubated at different temperature ranging between 30- 90 ℃ at an interval of 10 ℃. The enzyme activity was carried out according to the standard assay procedure.

2.2.6.3 Effect of Metal ion on the Activity of Partially Purified Enzyme

The effect of different metal ions on the activity of the purified enzyme at 5 mM and 10 mM were determined using Mg^{2+}, Ca^{2+}, AL^{3+}, Cu^{2+}, Fe^{2+}, Zn^{2+}, K^{2+}. The substrate was pre-incubated with the above listed metals and enzyme activity was carried out using the standard assay procedure.

2.2.6.4 Effect of Inhibitor on the activity of Partially Purified Xylanase

The effect of different Inhibitors on the activity of the purified enzyme at 5 and 10mM were determined using L-cysteine, dithiothreitol, sodium dodecyl sulfate, mercaptoethanol, Triton X-100, Tween 20, Urea and EDTA. The substrate was pre-incubated with the inhibitors and enzyme activity was carried out using the standard assay procedure.

2.2.6.5 Measurement of Kinetic Constant

The kinetic constants, K_m and V_{max} of the purified xylanase were determined using Lineweaver-Burk plot (1934) by measuring the xylanase activity at different concentrations of substrate (carboxylmethyl cellulose) from 0.1- 0.8 % prepared in 0.02 M citrate buffer (pH 5.3). The enzyme activity (initial velocity) was determined according to the standard assay procedure. Then, inverse of initial velocities (V^{-1}) and substrate concentrations ($[S]^{-1}$) of each concentration were plotted. However, V_{max} and K_m were calculated using Lineweaver-Burk plot.

2.3 STATISTICAL ANALYSIS

All experiments were conducted in triplicate, and data sets were analyzed using Microsoft Excel 2016. Mean values were calculated and recorded alongside standard deviations (Mean $^+$ STDEV).

3.0 RESULTS

3.1 Seed culture

Plate of xylanase from *Fusarium solani* grown on seed culture (potatoe dextrose broth) is presented in plate 1.

Plate 1. *Fusarium solani* grown on Seed Culture

3.2 Activity of xylanase produce from *Fusarium solani*

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Xylanase activity and protein concentration results on the crude enzyme were found to be 5.09 units/mL and 0.77 mg/mL respectively.

3.3 Partial purification of xylanase from *Fusarium solani*

The summary of the partial purification of xylanase from *Fusarium solani* is presented in Table 1. The d dialysate obtained after dialysis of precipitated protein using ammonium sulphate showed yield of 4.56 % with 39.9-fold purification. When the dialysate was loaded on DEAE sephacel (ion exchange chromatography), a sharp peak activity was observed with 21.9 % recovery and 112.1 purification fold.

Table 1: Purification Table of Xylanase from *Fusarium solani*

Step	Volume (ml)	Enzyme Activity (U/ml)	Protein Conc (mg/ml)	Total Activity (U)	Total Protein (mg)	Specific Activity (U/mg)	Yield (%)	Purification Fold
Crude Enzyme	410	5.09	0.77	2086.9	315.7	6.61	100	1
$(NH_4)_2SO_4$ Precipitation	80	1.19	0.45	95.2	36	2.64	4.56	39.9
DEAE Sephacel	50	8.97	1.21	448.5	60.5	7.41	21.5	112.1

Total activity (mg) = Activity in the fraction (U/ml) × Total volume (mL)

Total Protein (U) = Protein concentration (mg/ml) × Total volume (mL)

Specific Activity (U/mg) = Total activity (U) / Total protein (mg)

Yield (%) = (Total activity of purified step / Total Activity of the crude) × 100

Purification fold = (Specific activity of purified Step / Specific Activity of the Crude) × 100

4.4 Characterization of Partially Purified Xylanase

4.4.1 Effect of pH on the Activity of xylanase from Fusarium solani

The effect of pH on the activity of partially purified xylanase from *Fusarium solani* is presented in Figure 1. The partially purified xylanase was found to be active in all the pH investigated. The relative activity increased from 40.01 % to 100 % between pH 2.0-4.0 while the pH optimum was observed to be pH 4.0. However, a gradual decline in relative activity was observed from pH 5.0-12.0 with a relative activity of 0 % at pH 12.0.

4.4.2 Effect of Temperature on the Activity of Partially Purified Xylanase

The effects of temperature on the activity of partially purified xylanase produced from *Fusarium solani* is presented in Figure 2. The enzymatic activity of partially purified xylanase increases as temperature are increasing up to 40 ℃ where optimum activity was at 40 ^{O}C. However, a minimum relative activity of 50.5 % and 23.5 % was obtained at 80 and 90.

4.4.3 Effect of Metal Ion on the Activity of Partially Purified Xylanase

The effect of various metal ions at 5 mM and 10 mM concentration on the activity of partially purified xylanase is presented in Figure 3. Al^{3+}, Cu^{2+}, Zn^{2+}, Hg^{2+}, K^{+}, Al^{2+}, Fe^{2+}, Ca^{2+} were observed to stimulate xylanase activity at all concentration except Mg^{2+} which inhibited the activity of xylanase while at 10 mM Zn^{2+}, Fe^{2+}, Cu^{2+}, Mg^{2+}, K^{+}, Ca^{2+}, Al^{3+} were all moderately activated.

4.4.4 Effect of Inhibitor on the Activity of Partially Purified Xylanase

The effect of inhibitors on the activity of partially purified xylanase is presented in Figure 4. The results clearly indicated that EDTA exceptionally inhibited activity at 5 Mm, but urea, sodium azide, β-mercaptoethanol, SDS, cysteine, Tween 20, Tween- X moderately modulate it at 10 Mm. While at 5 Mm EDTA, SDS and Tween 20 exceptionally inhibited a urea, sodium azide, mercaptoethanol, cysteine and Tween-X were moderately inhibited.

4.4.5 Kinetic Analysis

Lineweaver- Burk of partially purified xylanase is presented in Figure 5. The K_m and V_{max} values of xylanase were determined to be 0.37412 mM and 0.5362 mg/ml/min.

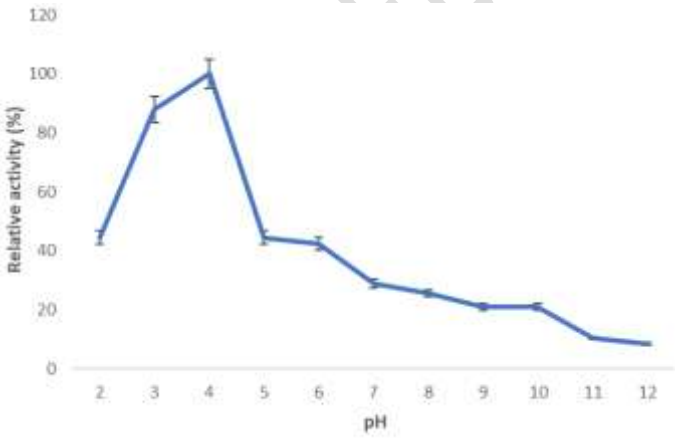

Figure 1: Effect of pH on the activity partially purified Xylanase

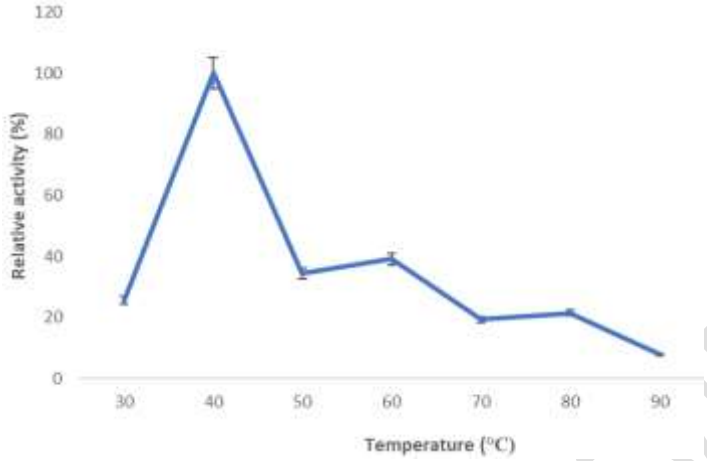

Figure 2: Effect of Temperature on the Activity of Partially Purified Xylanase

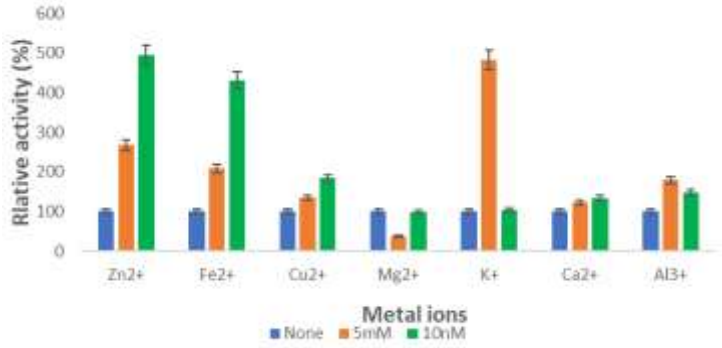

Figure 3: Effects of Metal ions on the Activity of Partially Purified Xylanase

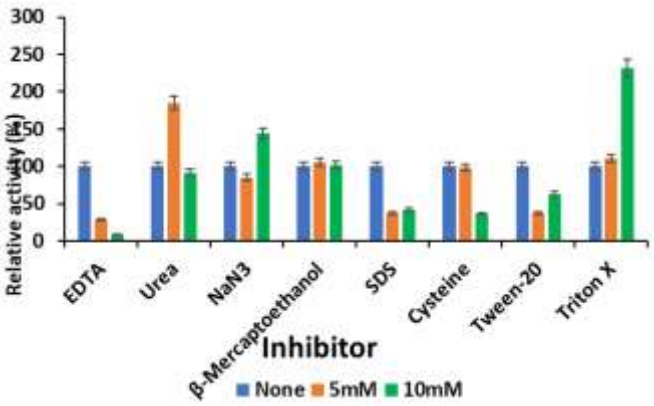

Figure 4: Effects of Inhibitors on the Activity of Partially Purified Xylanase

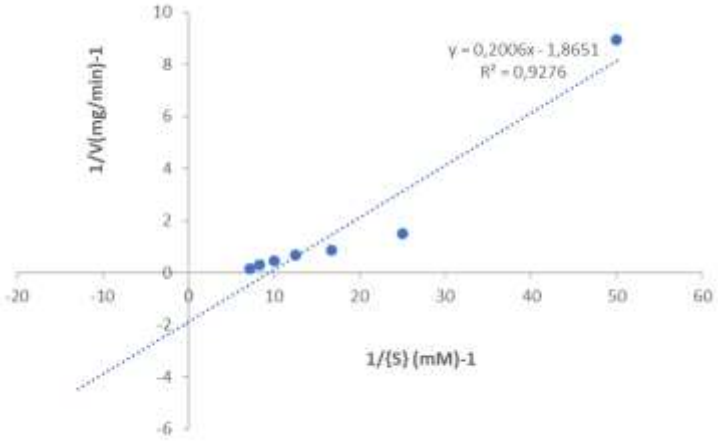

Figure 5: Kinetic parameters of the Partially Purified Xylanase

3.0 DISCUSSION

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The research focuses on the production, purification, and characterization of xylanase from *Fusarium solani*, isolated from the long-horned beetle. This fungal strain shows promising potential for producing xylanase, a key enzyme for breaking down hemicellulose. The enzyme is purified using techniques like ammonium sulfate precipitation and ion-chromatography. Characterization reveals its optimal activity at specific pH and temperature ranges, demonstrating stability and efficiency for industrial applications. This study emphasizes *F. solani* as a valuable source for eco-friendly and cost-effective xylanase production, useful in the feed, biofuel, and paper industries (Kumar & Satyanarayana, 2018).

The partial purification of xylanase is a pivotal step in enzyme biotechnology, especially for its use in the food and biofuel industries. In Table 1, ammonium sulfate precipitation yielded 4.56% and a purification fold of 39.9. Almeida et al. (2022) reported a higher purification fold of 93.41 and a yield of 4.49 for xylanase from *Bacillus pumilus*, using additional purification methods post-precipitation. Furthermore, Bharti et al. (2023) validated the effectiveness of ammonium sulfate precipitation with *B. pumilus*. Subsequent purification typically involves ion exchange chromatography, such as DEAE Sephacel, which in Table 1 resulted in a 21.5% recovery and an 11.23-fold purification, aligning with (Bangaru et al., 2022), who combined both methods for enhanced xylanase purification. Bangaru et al. (2022) further underscored the critical role of chromatography in minimizing contaminants. Variability in purification success arises from microbial sources, as seen with xylanase from *Aspergillus oryzae*, which achieved a 38% yield and a 36.97 purification fold, outperforming the results in Table 1 as reported by Adhyaru et al. (2017). This variability underscores the influence of the enzyme's source on purification outcomes.

The effect of pH on the activity of partially purified xylanase is shown in Figure 1. The enzyme was active across all pH levels tested, with its relative activity increasing from 40.01% to 100% between pH 2.0 and 4.0, reaching its optimum at pH 4.0. However, a gradual decline in activity occurred from pH 5.0 to 12.0, with no activity observed at pH 12.0. The effects of temperature on the activity of partially purified xylanase are presented in Figure 2. The enzymatic activity increases with rising temperature, reaching its optimum at 40°C. At higher temperatures, the activity decreases, with minimum relative activities of 50.5% and 23.5% observed at 80 and 90℃, respectively. This pattern aligns with findings from other studies. For instance, xylanase from *Anoxybacillus kamchatkensis* showed optimal activity between 50 and 70°C, highlighting that many xylanases perform efficiently at elevated temperatures, which is beneficial for industrial applications (Yadav *et al*., 2018). Similarly, Fang et al. (2022) reported that endoxylanases generally exhibit enhanced activity within the 40-80°C range, emphasizing the importance of thermal stability in high-temperature industrial processes. While the xylanase in Figure 2 shows peak activity at 60°C, other xylanases can maintain activity at even higher temperatures, suiting them for specific uses. Structural alterations, such as N-terminal and C-terminal truncations, can significantly enhance the thermal stability and catalytic efficiency of xylanases (Bhattacharya, et al., 2020). This underscores the influence of the enzyme's amino acid sequence on its thermal stability. (Bharti, et al.,

2023; Jacomini, et al., 2023) further noted that the optimum temperature for xylanases varies among bacterial strains, with many showing peak activity between 40 and 50°C. In contrast, (Amobonye *et al*., 2021) found that xylanase from *Beauveria bassiana* exhibits optimal activity at lower temperatures (around 35°C), demonstrating that not all xylanases are thermophilic. This variability in optimal temperatures underscores the need for careful selection of xylanases based on their thermal profiles for industrial applications.

The effect of various metal ions at 5 and10 mM concentrations on the activity of partially purified xylanase is shown in Figure 3. Ions such as Al^{3+}, Cu^{2+}, Zn^{2+}, Hg^{2+}, K^{+}, Al^{2+}, Fe^{2+}, and Ca^{2+} were found to stimulate xylanase activity at both concentrations, except for Mg^{2+}, which inhibited its activity. At 10 mM, Zn^{2+}, Fe and K^{+} showed a pronounced activating effect while Cu^{2+}, Ca^{2+} and Al^{2+}showed moderate activation. Kumar et al. (2023) and García-Depraect et al. (2022) similarly reported that xylanase from *Bacillus paramycoides* displayed increased activity in the presence of Co^{2+}, Ca^{2+}, Mg^{2+}, Zn^{2+}, and Fe^{2+}, suggesting that certain ions can act as activators. Likewise, Bhattacharya et al. (2020) reported that xylanase from *Nectria haematococca* was activated by Mn^{2+} and Zn^{2+}, while Cu^{2+} had a concentration-dependent effect stimulating the enzyme at lower concentrations but inhibiting it at higher ones similar to the findings on Zn^{2+} in Figure 4.

The impact of inhibitors on the activity of partially purified xylanase is essential for understanding how different substances influence enzyme performance in industrial settings. As shown in Figure 4, surfactants and inhibitors affected xylanase activity at 5 mM and 10 mM concentrations. At 5 mM, inhibitors like urea, β-mercaptoethanol, and Tween-20 significantly reduced the xylanase activity, while EDTA, SDS, cysteine, and Tween-X caused moderate inhibition. However, at 10 mM, SDS and cysteine enhanced activity, while β-mercaptoethanol and Tween-X showed strong inhibition. Studies provide valuable insights into these effects. Tundo et al. (2020) highlighted that xylanase inhibitors such as TAXI and XIP can bind to the active site of xylanases from species like *Aspergillus* and *Bacillus*, blocking substrate access and reducing activity. Similarly, β-mercaptoethanol's inhibition, seen in Figure 5, it is likely due to its interaction with thiol groups in the enzyme. Liu et al. (2021) emphasized that plant-derived inhibitors can significantly reduce xylanase efficiency, especially in food processing and animal feed industries. These findings highlight the importance of selecting enzyme formulations that can mitigate inhibitory effects, ensuring optimal xylanase performance in industrial applications where inhibitors may be present. (Abdella *et al*., 2021) further found a reduction in xylanase activity when EDTA was present, emphasizing the critical role of metal ions in maintaining enzyme stability. Inhibition by specific ions could result from their interaction with the enzyme's active site or structural changes, as discussed by Khalil et al. (2022) regarding heavy metal ions' nonspecific binding.

The Lineweaver-Burk plot is a key tool in enzyme kinetics, used to determine the Michaelis-Menten constant (Km) and maximum reaction velocity (Vmax). In this study, the purified xylanase showed a Km of 0.37412 mM and a Vmax of 0.5362 mg/ml/min, indicating its substrate affinity and catalytic efficiency. A

lower Km value (0.014279 mM) suggests a high affinity for the substrate, as the enzyme reaches half its maximum velocity at lower concentrations. This supports findings by García-Depraect et al. (2022), who noted that xylanase Km values vary based on purification methods and substrates. Menezes et al. (2023) similarly observed that Km values differ across xylanase sources due to structural differences. The Vmax of 1.472 mg/ml/min reflects the enzyme's maximum reaction rate. Additionally, the catalytic efficiency (kcat/Km) is crucial for enzyme assessment, with higher values indicating better efficiency, as demonstrated by Zhang et al. (2023) and Srivastava et al. (2022), highlighting the importance of kinetic parameters for industrial applications.

CONCLUSION

This research investigated the production, purification, and characterization of xylanase from *Fusarium solani*, isolated from the gut of longhorn beetles. The presence of xylanase activity in this fungal isolate is consistent with previous findings. These results suggest that this xylanase, derived from beetle-associated *Fusarium solani*, holds significant potential for industrial biotechnology applications. Key findings on enzyme purification, stability, metal ion effects, and kinetics align with and expand upon existing xylanase research. The enzyme's high efficiency in hydrolyzing xylan positions it as a promising candidate for use in industries such as food, feed, and biofuel production. Furthermore, this study emphasizes the potential of insect-associated fungi as a valuable and underexplored resource for industrial applications (Patel *et al*., 2021). Future research could explore genetic engineering strategies to enhance xylanase production, stability, and functionality for broader industrial use. Overall, this study advances our understanding of the ecological relationships between beetles and fungi and their potential contributions to sustainable biotechnology innovations.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Authors hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

DECLARATION OF COMPETING INTEREST

Authors report that there are no competing interests to declare.

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