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ORIGINAL RESEARCH PAPER

Isolation, Characterization, and Partial Purification of a Thermostable Alkaline Xylanase from Aspergillus sp. MS8

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Abstract

Technology Area

This technology falls under Industrial Biotechnology, specifically focusing on enzyme technology and bioprocessing for lignocellulosic biomass valorization, with applications in industrial enzyme production and sustainable biorefinery processes.

Technology

The present technology relates to the isolation, screening, production, and partial purification of a thermostable and alkaline xylanase enzyme from a fungal strain identified as Aspergillus sp. MS8, isolated from soil and lignocellulosic waste samples collected from Maharshi Dayanand University Forest, Rohtak, Haryana, India.

A total of 39 fungal isolates were obtained and screened using qualitative (Congo red assay) and quantitative (DNSA method) approaches for xylanase activity. The selected isolate MS8 demonstrated the highest enzyme activity and was further characterized morphologically and biochemically.

The enzyme was produced under submerged fermentation using Czapek's medium and partially purified via acetone precipitation, resulting in a ~3-fold increase in specific activity. SDS-PAGE analysis indicated a molecular weight of approximately 25 kDa, and zymogram analysis confirmed enzyme purity and absence of isoforms.

Potential Market / Customers

This technology has significant applications across multiple industrial sectors, including: Pulp and Paper Industry: Biobleaching and delignification processes, Biofuel Industry: Pre-treatment and saccharification of lignocellulosic biomass for bioethanol production, Food and Feed Industry: Improvement of digestibility and nutritional value of animal feed

Technology Valuation (Commercial Potential & Value Proposition)

The developed xylanase enzyme demonstrates strong commercial potential due to the following value propositions:Thermostability and Alkaline Tolerance: Suitable for harsh industrial processing conditions, reducing enzyme denaturation and increasing process efficiency.

INTRODUCTION

Xylan, major plant hemicellulosic material, make-ups the cell wall of plants (Walia et. al, 2017). Xylan is an excellent fiber of great consistency and integrity, present in the hardwood and softwoods of plants (Kumar et. al., 2017; Shankar et. al., 2018). Xylan is made up of the chain of 1, 4-β-D xylopyranosyl having different functional groups. The polymerization levelof xylan varies from hardwood to softwood and it can be used in various valuable products. Xylan is a complex compound and cannot degrade easily and the enzymes responsible for its degradation are called as xylanases. Its degradation requires the combined action of various enzymes (Rao and Li, 2017). The central straight chain is cleaved by endo-1, 4-β-D-xylanases at random sites and β-D-xylosidases cause the removal of xylose monomers from the non- reducing ends ofxylobiose or xylo-oligosaccharides chain. Other side groups attached withthexylan chain are removed via the action of acetyl xylan esterases, α-D-glucuronidases, and α-L-arabinofuranosidases, etc. (Kumar and Shukla, 2015).

Xylanases are the enzymes which act on the substrate xylan, responsible for the hydrolytic cleavage of β-1, 4-glycosidic bonds in xylan. As the enzyme cleaves the xylan at the β -1, 4 Xylanase has various potential applications in different industries such as food industries, textile in¬dustries, paper industries, animalfeed industries, juice industries, brewing industries, sites, called as endo-1, 4-β-xylanase. Xylanases are also known with various other names such as endoxylanase, β-xylanase, β-1, 4-xylanase, β-1, 4-D-xylan-xylanohydrolase, pentosanases, andendo-1,4- β-D-xylanase (Basu et al., 2017). Xylanases were first reported in 1955 and enzyme code was assigned in 1961 by the International Union of Biochemistry and Molecular Biology(IUBMB). The enzyme code of xylanase is EC 3.2.1.8 (Kumar and Shukla, 2016)

Microorganisms are the major source of xylanases which includes bacteria, fungi, yeasts, and actinomycetes. Bacterial genera known to produce xylanase are *Bacillus, Cellulomonas, Micrococcus, Pseudoalteromonas, Caldicoprobacteralgeriensis*, etc. Fungal genera known to produce xylanases are *Aspergillus, Fusarium, Penicillium, Trichoderma, Phomopsis, Cunninghamellaelegans,* etc. Fungal xylanaseswithhightemperaturestability, coldadaptivity, or alkali-stability have been isolated and purified;*Trichoderma reesei* (mesophilic), *Cladosporium* sp. (cold-active), *Thermotoga* sp. (thermostable), *Neocallimastix frontalis* (acidic), *Gloeophyllumtrabeum* producesxylanase at hightemperature (70°C) under broad pH range (4-7) (Rao &Li, 2017).

bioremediation and bio-refinery for forest and agriculture wastes (Ramanjaneyulu, 2015,Kumar et. al., 2017). Initially, xylanases were used in the preparation of animal feed but

lateron applied in the various other fields such as paper and textiles. Xylanases together with cellulases and pectinases account for approximate 20% of the world's enzyme market. Xylanases are used in the baking industries to accelerate the dough preparation process and in animal feed preparationwhere they aid inthe digestion(Aliet. al., 2014). Nowadays xylanases are also applied in the paper and pulp industries, saccharification of lignocellulosic materials, biofuel industry and organic waste treatment plants.

Due to vast applications of xylanases, the need of isolation and screening of xylanaseproducing microorganisms forthe productionofxylanases increasing daybyday(Kumar et al., 2016; Kumar et al., 2017). The production ofxylanases can be done byboth submerged (SmF) and solid-state fermentation (SSF) processes (Nair et. al, 2008). The submerged fermentation (SmF) technique is widely used for the production of enzymes due to its ease in operation.

This study was aimed to isolate the xylanase producing fungi. Various soil samples are collected from the different sites of the MDU, Rohtak campus for the isolation of fungus and screened for the production of xylanases. Further, the enzyme was produced by using isolated fungal species and purified for the characterization. This study was aimed to isolate the xylanase producing fungi. Various soil samples are collected from the different sites of the MDU, Rohtak campus for the isolation of fungus and screened for the production of xylanases. Further, the enzyme was produced by using isolated fungal species and purified for the characterization. The present investigation has been planned with the objectives of isolating and screening xylanase-producing microorganisms, which includes the collection of soil samples, isolation of fungi using the serial dilution method, and screening of the isolates for xylanase production. Further, the study aims to produce xylanase through submerged fermentation, followed by purification of the enzyme and subsequent characterization of the purified enzyme.

Methodology

Samplecollection

Soil samples were collected fromMaharshi Dayanand University, Rohtak forest in polythene bags, labeled properly and processed for the isolation of fungi.

Table3.1:List ofdifferentsamplescollectedMaharshiDayanand University, Rohtak.

Sr.No.	Typeofsample collected	Samplecode
1.	Soilsample	SoilSample(SS)
2.	Decayingwood 1	Round wood (RW)
3.	Decayingwood 2	Squarewood(SWA)
4.	Decayingwoods oil	Square wood(SWU)

Isolationoffungi

For the isolation of fungi from soil samples, serial dilution technique was used.For this 1 gsoil was weighed and suspended into 10 mL autoclaved distilled water blank. From this stock suspension, 1mL of sample was serially diluted into 9mL of sterile distilled water blanks up to 10^{-5} 100 µL samples $10^{-3}$to 10^{-5}were then plated on Potato Dextrose Agar (PDA) plates in duplicates. The plates were then incubated at 28°C - 30°C for 3 days and observations were made daily to determine the presence of fungi. Pure cultures of isolates were obtained by repeated sub-culturing on PDA.

Screeningofxylanaseproducing fungi

Qualitativescreeningofisolatesforxylanase production

All the fungal isolates were processed for qualitative analysis xylanase production during their growth. All the fungal isolates were inoculated on the Czapek's agar medium containing beechwood xylan as the sole source of carbon source at the 0.1% (w/v) concentration.After inoculation, plates were incubated for 3 days at 30°C. After the incubation time, plates were flooded with 0.1% (w/v) congo red staining solution for 30 min and washed with 1 M NaCl solution to observe the zone of clearance around the fungal growth. The fungal strains, which showed growth on Czapek's agar medium with xylan and the clear zone around the colonies were selected as positive xylanase producers.

Quantitativescreeningofisolatesforxylanasep roduction

For this purpose, 50mL of liquid czapek's mediumwith1% (w/v) beechwoodxylana carbon source was prepared and inoculated with 1 mL spore suspension from five days old fungal culturegrownonPDAslant. Submerged fermentation(SmF)wasperformedat 30˚Cfor7days. After 7 days of incubation, the crude enzyme was extracted by filtration and the filtrate was centrifuged at 12,000 rpm for 10 min at 4˚C. The cell-free culture filtrate (CFCF) was used as the crude enzyme.

Xylanase activity was measured by xylanase assay, which was carried out at 55°C for 10 min using 1% (w/v) beechwood xylan (HiMedia Laboratories Pvt. Ltd. Mumbai) as reaction substrate in 0.05M sodium citrate buffer, pH 5.3.

Xylanaseassay

The xylanase activitywas calculated according to the method proposed byBailey et al. (1992). Quantification of reducing sugar has been done using the DNS method reported by Miller,1959 using xylose (HiMedia Laboratories Pvt. Ltd. Mumbai) as standard. CFCF was used asthe crude enzyme. A reaction mixture was prepared by mixing 0.5mL 1% beechwood xylanand 0.5 mL diluted enzyme. The reaction mixture was incubated for 10 min at 60°C. The reaction was stopped by adding 1 mL of DNS reagent and was then boiled for 20 min. The

reaction mixture was allowed to cool and then 0.2 mL of 33% sodium- potassium tartrate solution was added. Absorbance was measured at 540 nm using UV-Vis spectrophotometer Enzyme units of xylanase activity can be defined as micromoles (µmol) of xylose produced by one mL of the enzyme in one minute during the enzyme-substrate reaction.

Platewelldiffusionassay

Plate well diffusion assay was performed to qualitatively analyze enzyme activity. Czapek's agar medium plates containing 1% beechwood xylan medium was poured in sterilized petri platesandwereprepared. Asinglewellwas madeineachplatebyusingsterilizedcorkborerof 10 mm diameter. To each well, 100 µL of the CFCF was poured and incubated at 30˚C overnight. After 24 hours, incubated plates were stained with 1% Congo red dye for 30 min.

Afterward,stainedplateswerefloodedwith1MNa Clfordestaining.Here,theappearanceof clear zones confirmed the presence of 1-4-β-endo-xylanase activity.

Characterization ofmorphologicalfeatureofisolates

The fungal isolates were identified based on the basis of their cultural and morphological characteristics. The cultural characteristics were determined by their appearance on culture plates while the morphological features were determined microscopically.

Enzymeproduction andpurification

The fungal strain showing the highest xylanase activity was cultured for 7 days for production of xylanase in production medium under submerged conditions. After 7 days, enzymebroth was harvested by filtration through Whatman filter paper and then centrifuged at 10,000 rpmfor 10 min at 4℃. The enzyme was concentrated by adding chilled acetone followed by overnight incubation at-20℃. After precipitation, centrifugation was done at 12,000 rpm for20 min and the pellet was dissolved in minimum amount of citrate buffer (0.05M; pH 5.3).

Estimationoftotalproteincontent

The estimation of total protein content present in the sample was quantified by using Lowry's method taking bovine serum albumin (BSA) as standard.For this purpose, 1 mL of protein sample to be quantified was taken in a test tube in duplicate and 5 mL of solution 1 was added to each test tube and incubated at 30℃ for 10 min. After that, 0.5 mL of solution 2was added to each test tube and the tubes were then incubated in dark for 30 min at 30℃. After 30 min absorbance of each reaction mixture was measured at 660 nm.

Determinationofthepurityofenzyme

Polyacrylamidegelelectrophoresis(SDS-PAGE)andzymogramanalysis

To investigate the purity of enzyme and to visualize the protein, Polyacrylamide gel electrophoresis (PAGE) was carried out after purification of xylanase. 12% SDS PAGE (Laemlli, 1970) and 10% native PAGE

(Ornstein and Davis, 1964) was prepared. In both the gels approximately20 µL ofthe sample was loaded and runat 70Vtillthe loading dye reaches to the bottom of the gel. After running, the SDS PAGE gel was stained with Coomassiebrilliant blue-R-250(CBB-R-250)anddestainedwiththedestainingsolutionuntilthebandsare visible.

For zymogram, 10% native gel with 1% (w/v) xylan was prepared. After running, the gel with 1%xylanwas incubated at 55°Cin0.05 Mcitrate buffer, pH 5.3 (Royer and Nakas, 1990) for 30 min to hydrolyze xylan. After that, the zymogram was stained with 0.1% congo red for 30 min and destained with 1 M NaCl solution for an hour.

Characterizationofpartiallypurified xylanase enzyme

Determinationoftemperatureoptima

The temperature optimum of the partially purified xylanase was determined. The partially purified xylanase was first diluted 100 times by adding citrate buffer (pH 5.3). To find out the temperature optima enzyme-substrate reaction was carried out at various temperatures 25°C, 35°C, 45°C, 55°C, 65°C and 75°C.The reaction mixture containing 0.5mL of substrate solution (1% xylan in citrate buffer) and 0.5 mL diluted partially purified xylanase were prepared and incubated at different temperatures for 10 min. The enzyme activities at different temperatures were assayed by DNSA method proposed by Miller, 1959.

DeterminationofpHoptima

The temperature optima of the partially purified xylanase were determined. The partially purified xylanase enzyme was first diluted 100 times by adding citrate buffer. To find out the pH optima enzymatic reaction was carried out at various pH ranging from pH 1.0 to pH 14.0. The reaction mixtures were prepared in different buffer solutions, hydrochloric-potassium chloride buffer (pH 1.0 – pH 2.0), citrate buffer (pH 3.0 – pH 6.0), phosphate buffer (pH 7.0 - pH 8.0), glycine-NaOH (pH 9.0- pH 10.0), sodium hydrogen orthophosphate-NaOH buffer(pH 11.0- pH 12.0), NaOH-KClbuffer (pH 13.0- pH 14.0). The same buffer was used to make the xylan substrate solution. The reaction mixture containing 0.5 mL of substrate solution (1% xylan) and0.5 mL diluted partiallypurified xylanase were prepared and incubated at 55°C for 10 min. The enzyme activities at different pH were assayed by DNSA method proposed by Miller, 1959.

Determinationofsubstratespecificity

To determine the substrate specificity ofthe partially purified enzyme different substrates were used in the reaction mixture like carboxymethyl cellulose (CMC), p-nitrophenyl xylopyranoside, starch, cellubiose, arabinogalactan, birchwood xylan. The reaction mixture containing 0.5 mL of substrate solution (1%) and 0.5 mL diluted partially purified xylanase were prepared and incubated at 55°C for 10 min. The enzyme activity was assayed

by DNSA method proposed by Miller, 1959.

Identificationofhydrolysisproducts

Thehydrolyzedproduct frombeechwoodxylanwasexamined bythin layerchromatography (TLC). Partially purified xylanase was incubated in 50 mM buffer (pH 5.2) containing 1 % beechwood xylan (Xy) at 55°C for different time intervals. After that, all the aliquots were spotted on the TLC plate and run using the mixture of butanol:ethanol:distilled water (5:3:2). Xylose (1mg/mL) was used as standards. The hydrolysates were detectedbyspraying withthe visualizingagent(methanol:sulphuricacid, 9:1) followedbyincubationat80°Cuntilthe spotsdeveloped ontheplate.

RESULTS

Isolationoffungalisolates A total of 39 fungal solates were isolated from various soil and lignocellulosic samples collected from the Maharshi Dayanand University, Rohtak forest. The fungal isolates were subcultured repeatedly and were maintained on PDA plates for further studies. Some fungal isolates have beendepicted inFig 4.3. The details ofvarious samples collected and the number of fungal isolates obtained has been mentioned in Table 4.1.

Screeningofisolatesforxylanase production

Qualitativescreeningofisolatesforxylanase production

Allthe 39 fungal isolates were grown on Czapek's agar mediumplates containing beech wood xylan as a sole source ofcarbon and it was observed that out of39 isolates, 10 isolates showed growth and zone of hydrolysis around the growth after flooding with congo red and destaining with 1M NaCl. These isolates were further processed for quantitative analysis for xylanase
production.

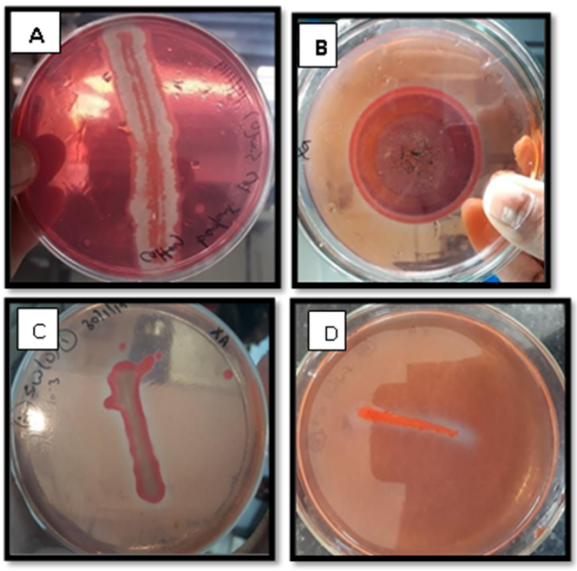


Fig 4.4:Qualitativeplate assayofisolates.
A. negative forxylanaseproduction,B,CandD.Positivefo rxylanaseproduction.

Quantitativescreeningofisolatesforxylanasep roduction

Standardcurvepreparation:

Standard curve of xylose (Conc. 5mg/mL as the stock solution) was prepared using the DNSA method (Miller, 1959).

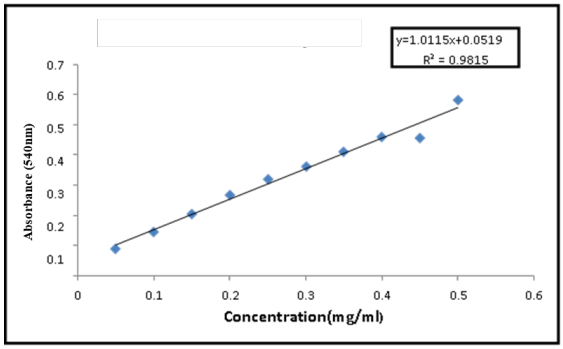


Fig4.5:StandardcurveofxyloseforXylanaseassay

Xylanaseassay

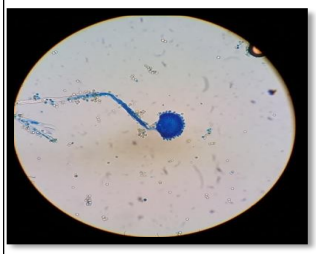
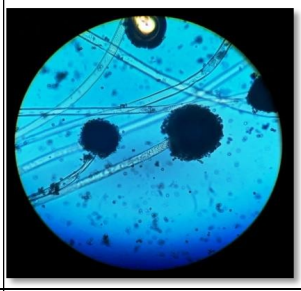
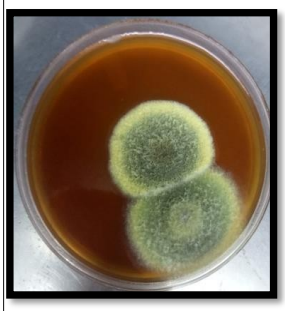
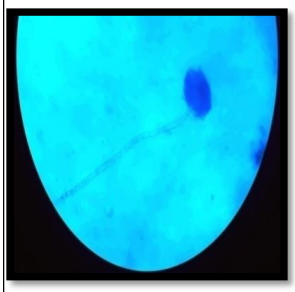
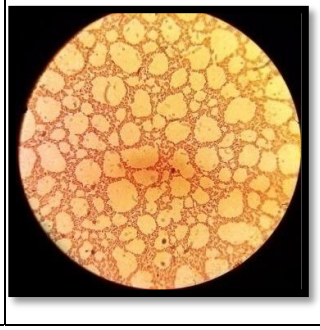
Out of 10 isolates selected in qualitative screening, 5 isolates were processed for quantitative analysis for xylanase production. The submerged fermentation was carried out using beechwoodxylanas a carbonsource. Xylanase activitywas estimated bycalculating the xylose concentration fromthe standard graph of xylose. Isolate MS8 was found to produce an enzyme with the highest xylanase activity, i.e, 112.8 U/mL. Other isolates MS6, MS12, MS13, MS36 showedxylanaseactivity,38.72,35.29,30.08,56.02U/mL,respectively(Table4.4)

Table4.4:Quantitativescreeningofselectedfungalisolates forxylanaseproduction.

Sr.No.	Isolate	Xylanaseactivity(U/mL)
1	MS6	38.72
2	MS8	112.8
3	MS12	35.29
4	MS13	30.08
5	MS36	56.02

Platewelldiffusion assay

Plate well assay was performed to qualitatively analyze the xylanase production in the fermentation medium. A clear zone was observed around the well inoculated with CFCF indicating the presence of 1-4-β-endo-xylanase activity.

Sr. No.	Isolate	Macroscopicview	Microscopicview	Result
1	MS6			Aspergillus sp. MS6
2	MS8			Aspergillus sp. MS8
3	MS12			Aspergillus sp. MS12
4	MS13			Aspergillus sp. MS13
5	MS36			Gram negative, smallrods, MS36

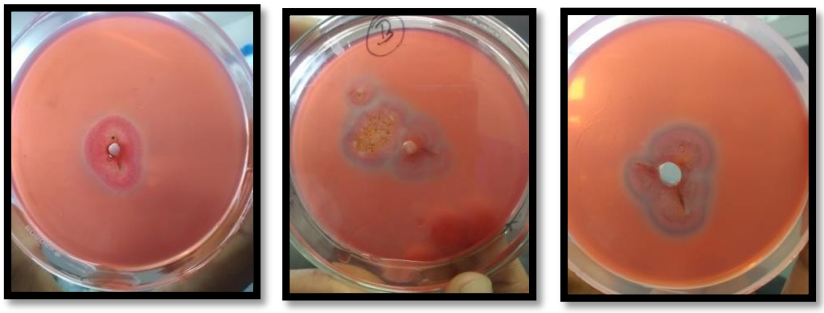


Fig4.7:Plate welldiffusion assayoftheharvestedcrudeenzymefromdifferentisolates.
A. MS6, B. MS8,C.MS12andD.MS13

Characterizationofmorphologicalfeatures ofisolates

Fungalisolateswerecharacterizedbasedonmacroscopicaswellas microscopicviews.

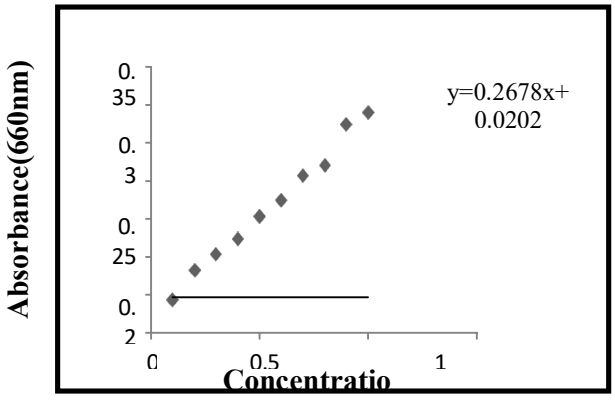
Table4.5:
Characterizationofisolatesbasedonmacroscopicand microscopic studies.

Enzymeproduction andpurification

Preparation of standard curve of Bovine Serum Albumin (BSA)

StandardcurveofBSAwasprepared according totheLowry'smethod (Fig4.8).

Fig4.8:Standard curveofBSAforproteinestimation



Xylanaseassayandtotalproteinestimationofth epartiallypurifiedenzyme.

The fungi showing the highest xylanase activity was further cultivated in 1L production medium under submerged conditions. The cell-free culture filtrate (CFCF) was precipitated by acetone precipitation method and it was observed that the after-acetone precipitation of CFCF, the xylanase activity increase by 1.37 folds and the specific activity increased by 2.78 (~ 3) folds. The table shows the purification step and data obtained from protein determination and xylanase activity assay results.

Table4.7:Purificationfor xylanasefromculturefiltrateofMS8.

Sr. No.	Purificationstep	Xylanaseactivity (U/mL)	Totalprotein (mg/mL)	Specific activity (U/mg)	Fold Purification
1	Crude	175.58	20.25	8.67	1
2	Acetone precipitation	241.0	10	24.10	2.78

Determining thepurity oftheenzyme

Aprominent bandwasobserved inthe18% SDS-PAGE runwiththepartially purified xylanase. The zymogram, on the other hand, showed a clear zone of hydrolysis against red background after staining with 0.1% (w/v) congo red and destaining with 1M NaCl.

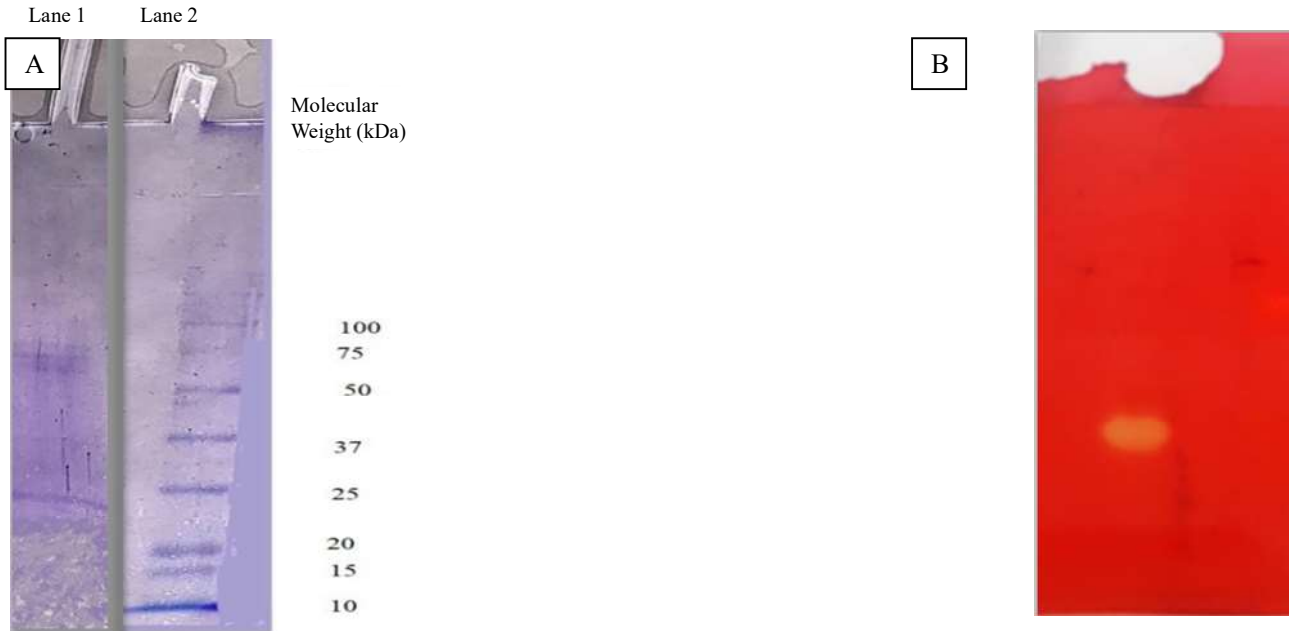


Fig 4.9: (A) SDS-PAGEofpartiallypurifiedxylanase.Lane 2 molecular marker. Lane 1 partiallypurifiedenzyme, (B) Zymogramofpartiallypurified xylanase.

Characterizationofpartiallypurifiedxylanase enzyme

Determinationoftemperatureoptima

The temperature optimum of the partially purified xylanase was found as 55℃. The enzyme showed lowxylanaseactivityat 25℃and35℃whereas it wasquiet highat temperature45℃, 65℃and 75℃. (Fig 4.10)

Determinationofsubstratespecificity

The activity of partially purified xylanase was determined with different substrates. Partially purified xylanase exhibited very high activity on xylan substrates, with the highest activity on birchwood xylan, while their activities on cellulose and p-nitrophenol substrates were negligible.

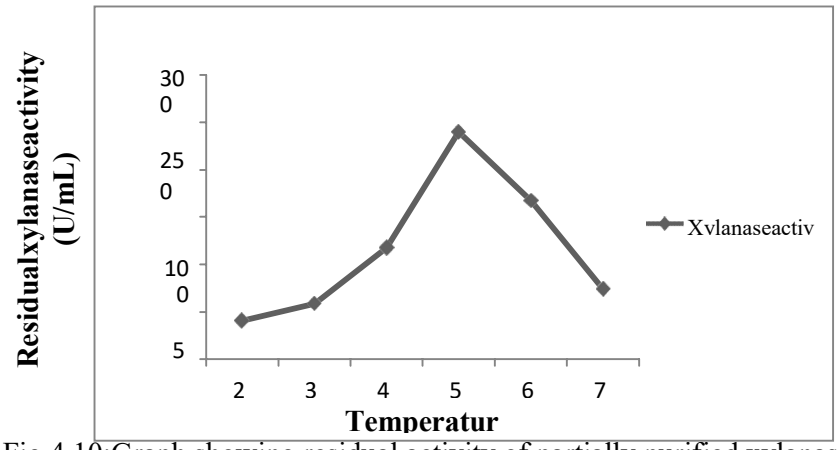


Fig 4.10:Graph showing residual activity of partially purified xylanase at different temperatures.

DeterminationofpHoptima

The partiallypurified xylanase showed highest activityat pH 8.The enzyme showed quiet high xylanase activityover a broad range, pH 5to 10 (fig 4.11)

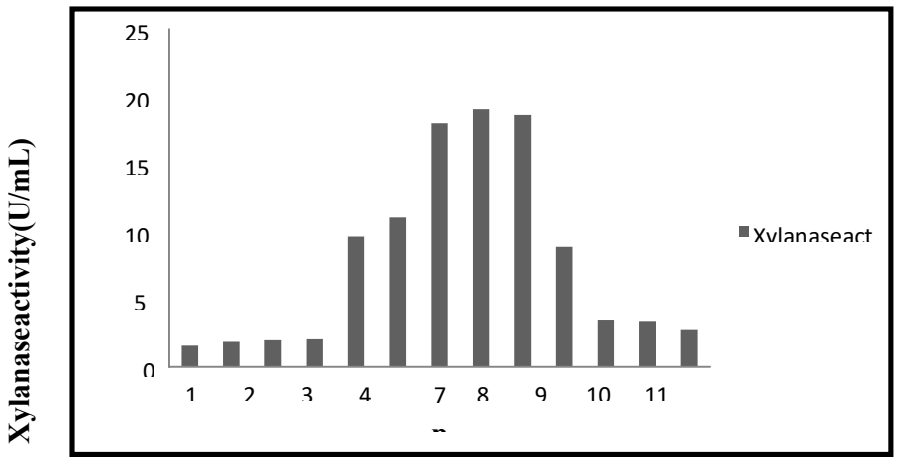


Fig4.11:Graphshowing residualactivityofpartiallypurifiedxylanaseatdifferentpH.

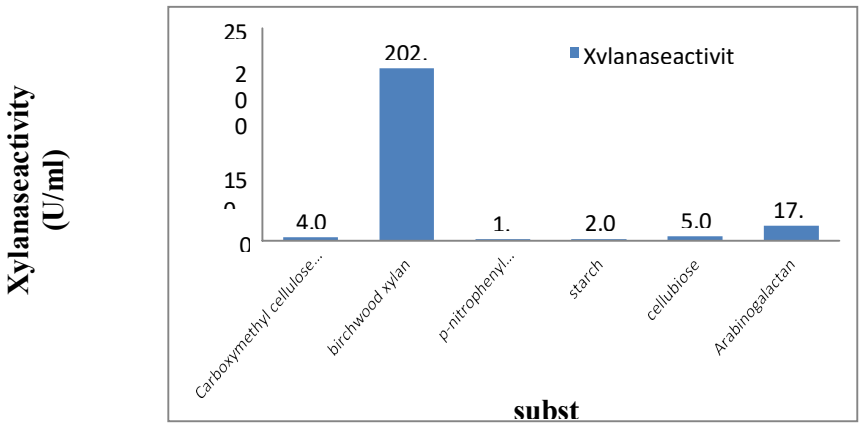


Fig 4.12: Graph showing xylanase activity of partially purified enzyme with different substrates.

Identificationofhydrolysisproducts

To identify the hydrolysis product of the enzyme-substrate reaction, TLC was performed. Itwas observed that the aliquots produce the spots at the same distance from the initial line asthatofstandardxylose. Thealiquotsalso producesmear beneaththespot equivalentto standard xylose and it was predicted that end product of xylan hydrolysis as xylose, and other xylooligosaccharide which has high molecular weight than xylose.

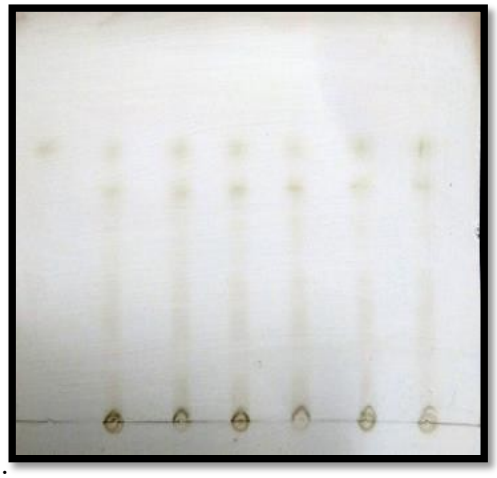


Fig 4.13:TLCanalysisofhydrolysisproductsofbeechwoodxylan bythepartiallypurified enzyme.
Fromleft toright,spot 1.Commercialxylose(1mg/mL),spot 2-7.Aliquotsofdifferent rection time (spot 2. 10min, spot3. 20 min, spot 4. 30 min, spot 5. 1hours, spot 6. 2hour, spot 7. 3 hours).

DISCUSSIONAND CONCLUSION

Xylanases are enzyme which acts on xylan, a hemicellulose, majorly found in the plant cell wall. Therefore, for isolation of xylanase producing fungi, soil sample and lignocellulosicwaste were collected from Maharshi Dayanand Universityforest, Rohtak, Haryana, India. Initially, 39 fungal isolates were obtained from soil and lignocellulosic wastes when grown PDA plates. The isolates were then screened through qualitative and quantitative analysis. For qualitativeanalysis, congo redassaywasused(Samantaet. al., 2011). Forthispurpose, isolates were grown on czapek's medium containing beechwood xylan as the sole source of carbon, flooded with 1% congo red and then destained with 1M NaCl. Congo red binds to xylan as it contains β-1, 4-glycosidic linkage and forms a strong complex with it (Samanta et. al., 2011). Therefore, if the fungal isolate has produced xylanase, it will degrade the xylan present in the medium and convert into xylose or xylooligomers, congo red will not be able to bind to that area and a clear zone appears when flooded with 1M

NaCl. It was observed that only a few of them showed a zone of hydrolysis.

For quantitative analysis, DNSA method given by Miller, 1959 was used as xylose released after the enzyme-substrate reaction is a reducing sugar. 3, 5-Dinitrocellulosic acid (DNSA) is a yellow color compound which detects the presence of a free carboxyl group (C=O). DNSA reacts withreducing sugar, get oxidized and convertedintothe reddish-brown color compound. Xylanase assay was performed according to the method given by Bailey. One unit of xylanase activity was defined as the amount of enzyme that liberates 1 micromole of xylose per minute under standard conditions (Kamble and Jadhav, 2012). Isolate MS8 showed the highest xylanase activity and was, therefore, used for further studies. The fungal isolates were characterized based on their macroscopic and microscopic view and isolate MS8 was found as *Aspergillus*sp. Although, xylanase production from*Aspergillus*sp. iswellrecognized but there is still a continued demand for new isolates. For xylanase to be used industry should fulfill certain characteristics viz., high xylanolytic activity, temperature tolerance, pH tolerance, and operational stability.

The fungal isolate was grown czapek's medium under submerged condition. After incubation time is over, the media was filtered through Whatman filter paper and then centrifuged. The cell-free culture filtrate was used as a crude enzyme. The crude enzyme was precipitated by acetone.Itwasobserved that thespecificactivityoftheacetonepurified enzymeincreasedby3 folds.The purification level was approximately similar to that demonstrated by Aswini et. al., 2013.

The SDS analysis of partially purified xylanase showed a prominent band with molecular weight of approximately 25kDa. Similar results were obtained with a strain of Aspergillus giganteus in 2004 by Fialho and Carmona (Fialho and Carmona, 2004). In 2008, a group of researchers purified xylanase with molecular weight of 35kDa (Lu et. al., 2008).

The zymogram analysis revealed that the enzyme was highly pure containing no isomers as only a single clear zone of hydrolysis against the darkened background was observed was staining with congo red and destaining with 1M NaCl.

The purified xylanase from Aspergillus sp. MS8 was highly active at 55℃. Xylanase from Aspergillus giganteus showed the highest activity at 50℃ (Fialho and Carmona, 2004). Xylanase from Aspergillus flavus produced a novel β-1,4-endoxylanase (designed as AfXynB) showed optimum xylanase production at 55℃ at 7.5 (Chen et. al., 2019). The pH optima of purified xylanase were 8. Bajaj and Abbass in 2011 purified the xylanase from Aspergillus fumigatus strain MA28 which has optimum temperature and pH at 50 °C and pH 8,respectively (Bajaj and Abbass, 2011) Approximately similar results were obtained with xylanase fungi *Aspergillus flavus*which showed optimum activity at pH 7.5 and the

enzyme was quite stable at pH range of 4.0-9.5 (Chen et. al., 2019). Xylanase from *Thermoascusaurantiacus* var. *levisporus* KKU-PN-I2-1 with a molecular weight of 27 kDa, showed maximumxylanaseactivityat pH9.0andattemperature60°C(Chanwichaet. al., 2015). Abdel et. al. showed maximum xylanase activity at 55-60°C and at pH 9(Abdel et. al., 2012).

The purified xylanase was highly substrate specific, showing specificity only to birchwood xylan and arabinogalactan which are part of the plant cell wall. The almost negligible activity was obtained in the enzyme sample incubated with CMC, cellobiose, indicating enzyme to be highly substrate specific.

The thin layer chromatography was performed to determine the end product of the enzyme-substrate reaction. A smear under the spot equivalent to standard xylose spots wereobserved The TLC revealed that the end product of the enzyme-substrate reaction and xylan hydrolysisas xylose, and other xylooligomers which has higher molecular weight than that of xylose. Similar results wereobtained bya groupofresearchers in2012 while working with *Aspergillus flavus* FPDN1 (Bhatt et. al. 2012).

Conclusion:

The present study successfully showed isolation, screening, production, purification, and characterization of the partially purified enzyme under operational cultural condition. Atotalof39 fungal isolates wereobtained fromthe sample collected fromMaharshi Dayanand University, Rohtak forest. Out of 39 isolates only 10 isolates showed xylanaseactivity in qualitative screening. The best 5 isolates which showed good xylanase activity under qualitative screening were proceeded for quantitative screening. The best isolate was identified as *Aspergillus* sp. MS8, showing maximum activity of 110 U/mL. The crude enzyme was then processed for purification by acetone precipitation. The acetone purified enzyme showed 3folds increase in the specific activity compared to the crude enzyme. The acetone purified enzyme wasused for partialcharacterizationofxylanase. The characterizationrevealed thatthe xylanase produced by isolate MS8, isolated from the lignocellulosic material collected from Maharshi Dayanand University, Rohtak forest as thermostable, alkalophilic and substrate-specific xylanase showing best activity at temperature 55°C and pH 8 which are essential prerequisites for the enzyme to withstand industrialconditionsand to be used inthe production of various commercial products.

References

Abdel-Monem, O. A., El-Baz, A. F., Shetaia, Y. M., & El-Sabbagh, S. M. (2012). Production and application of thermostable cellulase-free xylanase by Aspergillus fumigatus from agricultural wastes. Industrial Biotechnology, 8(3), 152–161. Industrial Biotechnology

Adhyaru, D. N., Bhatt, N. S., Modi, H. A., & Divecha, J. (2016). Insight on xylanase from Aspergillus tubingensis FDHN1: Production, high yielding recovery optimization through statistical approach and application. Biocatalysis and Agricultural Biotechnology, 6, 51–57. ScienceDirect

Adsul, M. G., Bastawde, K. B., & Gokhale, D. V. (2009). Biochemical characterization of two xylanases from yeast Pseudozymahubeiensis producing only xylooligosaccharides. Bioresource Technology, 100(24), 6488–6495.

Ali, S., Elahi, S. F., Jamil, A., & Ahmed, S. (2014). Xylanase production by Trichoderma species and its application in bread making. Journal of Agricultural Science, 6(2), 176–182.

Basu, M., Kumar, V., & Shukla, P. (2018). Recombinant approaches for microbial xylanases: Recent advances and perspectives. Current Protein and Peptide Science, 19(1), 87–99. PubMed

Bouacem, K., Bouanane-Darenfed, A., Boucherba, N., Joseph, M., Gagaoua, M., Hania, W. B., &Fardeau, M. L. (2014). Partial characterization of xylanase produced by Caldicoprobacteralgeriensis, a new thermophilic anaerobic bacterium isolated from an Algerian hot spring. Applied Biochemistry and Biotechnology, 174(5), 1969–1981. SpringerLink

Chakdar, H., Kumar, M., Pandiyan, K., Singh, A., Nanjappan, K., Kashyap, P. L., & Srivastava, A. K. (2016). Bacterial xylanases: Biology to biotechnology. 3 Biotech, 6(2), 150. PMC

Kumar, A., & Shukla, P. (2016). Microbial xylanases: An overview of structural and functional aspects. Critical Reviews in Biotechnology, 36(2), 241–253.

Kumar, V., & Shukla, P. (2015). Emerging strategies for implementing xylanases in the pulp and paper industry. Critical Reviews in Microbiology, 41(2), 196–207.

Kumar, V., Syal, P., & Shukla, P. (2017). Xylanases and their industrial applications: A review. Journal of Applied Biology & Biotechnology, 5(1), 1–11.

Nair, S. G., Sindhu, R., & Shashidhar, S. (2008). Fungal xylanase production under solid state and submerged fermentation conditions. African Journal of Microbiology Research, 2(4), 82–86.

Ramanjaneyulu, G., & Rajasekhar Reddy, B. (2015). Production and industrial applications of microbial xylanases. Journal of Biotechnology and Biomaterials, 5(4), 1–11.

Rao, M. B., & Li, J. (2017). Microbial xylanases and their industrial application in pulp biobleaching: A review. 3 Biotech, 7(1), 11.

Shankar, S., & Rhim, J. W. (2018). Effect of xylan on the mechanical and antioxidant properties of cellulose-based films. Carbohydrate Polymers, 181, 233–242. Carbohydrate Polymers

Walia, A., Guleria, S., Mehta, P., Chauhan, A., & Parkash, J. (2017). Microbial xylanases and their industrial application in pulp and paper biobleaching: A review. 3 Biotech, 7(1), 11. Springer