

Original Research Article

Xylanase-screening and biosynthesis from *Aspergillus tamarii*

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A B S T R A C T

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The productions of extracellular xylanase were carried out by using *Aspergillus tamarii* was evaluated under different fermentation parameters by employing solid state fermentation (SSF) method. The xylanase producers detected by the clear zone around the colony by simple plate assay method. *Aspergillus tamarii* is the potential strain among the fungal isolates. The xylanase syntheses were screened by employing different substrates by fermentation parameters. The optimum pH 5.0, temperature 35°C, moisture 65% and inoculum size 1.0 ml and it showed 5.77 IU

Introduction

Microbial enzymes have shown tremendous potential for different applications. Over the years due to their remarkable features enzymes have occupied the centre stage of all the biochemical and industrial processes. Enzymes can carry out their myriads of bio-chemical reactions under ambient conditions, which makes their use eco-friendly and often the best alternative to polluting chemical technologies. Enzymatic treatment provides the same level of output as is achieved through conventional methods that use harsh chemicals. The twentieth century saw an unprecedented expansion in the field of enzyme kinetics because new fields like microbiology and biotechnology have rapidly begun to gain ground. Therefore, usage of enzymes at various industrial levels has also gained momentum.

Xylan is the most abundant renewable non-cellulosic polysaccharide present on earth. It is a major constituent of plant cell walls and constitutes around 20-30 % of the dry weight of tropical hardwood and annual plants. Studies reveal that xylan forms an interphase between lignin and other polysaccharides. It is mainly present in the secondary cell wall and covalently linked with lignin phenolic residues and other polysaccharides such as pectins and glucans.

Xylanases catalyze xylan hydrolysis, the major hemicelluloses component in plant cell walls. The xylan structure, however, can differ greatly depending on its origin (Huisman *et al.*, 2000), but basically, xylan is a branched heteropolysaccharide constituting a backbone of β -1,4 linked xylopyranosyl units substituted with

arabinosyl, glucuronyl and acetyl residues (Shallom and Shoham, 2003). The hydrolysis of the xylan backbone is accomplished by endoxylanases (EC 3.2.1.8) and β -xylosidases (EC 3.2.1.37) along with a variety of de branching enzymes, that is, α -L-arabinofuranosidases, α -glucuronidases and acetyl esterases (Collins *et al.*, 2005).

Xylanases are extracellular enzymes produced by microorganisms such as bacteria (saprophytic and phytopathogenous), mycorrhizic fungi, and some yeasts. The enzyme is also found in protozoa, insects, crustaceans, snails, seaweed, and also seeds of plants during the germination phase in the soil (Wong *et al.*, 1988). However there are reports of Xylanases from fresh water mollusc (Yarnura *et al.*, 1997) and plant fruits (Yamaki and Kakiuchi, 1979a).

In present investigation we made an effort on isolation and screening of xylanase producers and also agro industrial wastes screening for the production of xylanase. Optimization of fermentation parameters like effect of pH, temperature and inoculum size.

Materials and Methods

Isolation and screening of Xylanase producing Fungi

The fungal strains were isolated from soil, rotten vegetables and wood chips samples. The fungal isolations were done by using Czapek-Dox media containing (g/l) Sucrose-30.0; Sodium nitrate-2.0; K_2HPO_4 -1.0, $MgSO_4 \cdot 7H_2O$ -0.5; KCl-0.5; $FeSO_4$ -0.01 and oat-spelt xylan-0.5

Screening of Xylanase producers

Screening of fungal strain by plate assay and tube assay were done. For fungi modified Czapek-Dox with xylan as a substrate xylanase were screened by inoculating the organism on the agar plate containing media composition, and incubated for 24-48 hrs at 35°C, then the plate was observed for cleared zone around the colony and also stained with Congo red (1% w/v) for 15 minutes and distained the plate with 1M NaCl (Figure.2).

Screening of agro industrial wastes for the production of xylanase by fungal isolate (SSF)

15 g of wastes like wheat bran, rice bran, wood chips, paddy husk, sugarcane waste and pigeon pea waste were moistened with 65% of moisture and autoclaved. 1.0 ml of *Aspergillus tamaris* KLD 2 (Figure.1) isolate was inoculated, pH 5.0 and incubated at 35°C for 96-120 h. The flasks were flooded with 10 ml 50mM tris-HCl buffer (pH 8.0), mixed well and filtered. The filtrate was centrifuged at 10,000 rpm for 10 min and the supernatant was used as enzyme source for assay.

Assay of xylanase

The xylanase activity was determined by measuring the release of reduced sugars from oat spelt xylan (1% w/v) by dinitrosalicylic acid method (Miller, 1959).

The enzyme solution (0.5 ml) and 0.5 substrate (xylan 1% w/v) along with 1 ml of buffer were taken in a test tube, the tubes were then allowed to stand at room temperature for 10 mins, 3ml of dinitrosalicylic acid was added to arrest

Figure.1 *Aspergillus tamari* grown of fungal media

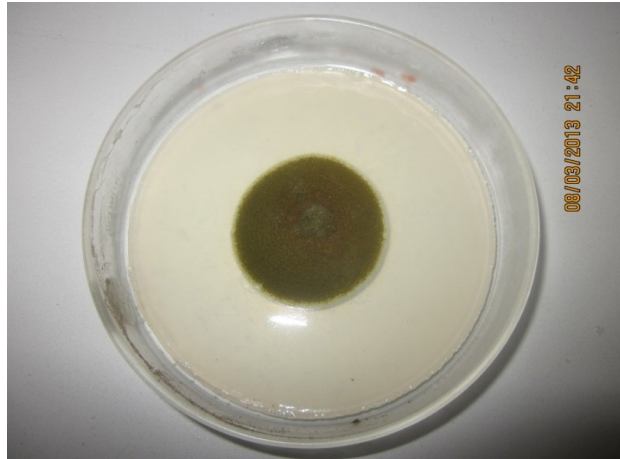


Figure.2 Plate assay of Xylanase producers

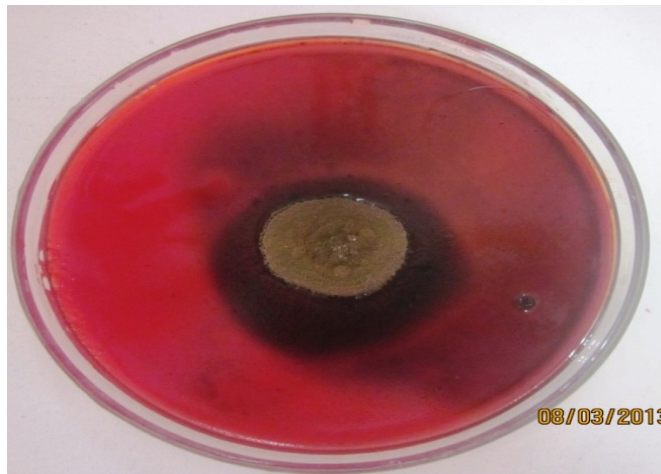
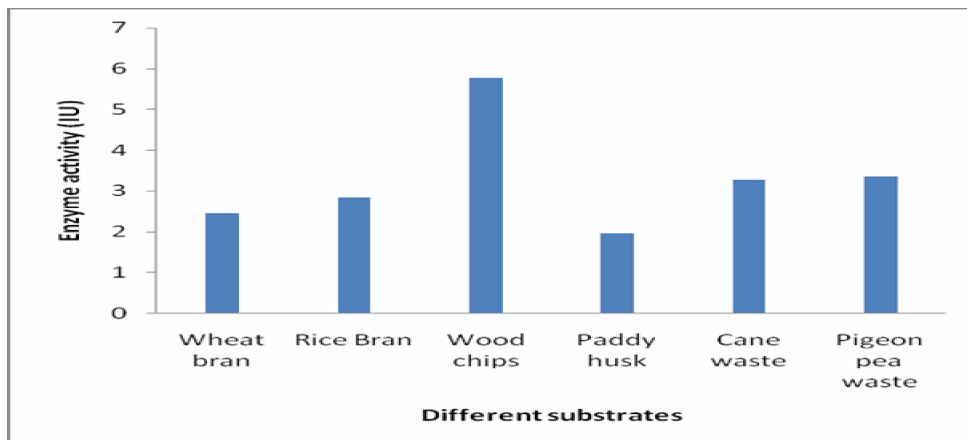


Figure.3 Screening of substrate for Xylanase



the reaction. After the addition of dinitrosalicylic acid, the tubes were placed in boiling water bath for 10 min. The color which had developed was read colorimetrically at 540nm. A blank test tube was prepared by adding dinitrosalicylic acid prior to the addition of enzyme to the test tubes. The enzyme activity was calculated by using the following formula; One unit of xylanase was defined as the amount of enzyme required to release 1 μ mol of xylose from oat spelt xylan in one minute under standard assay conditions.

Results and Discussion

Thirty *Aspergillus tammari* isolates were isolated from different sources such as soil, rotten vegetables and wood chips samples. All thirty isolates were named serially *Aspergillus tamarri* KLD1-KLD30 and used for screening of xylanase production by plate assay method. Out of thirty isolates *Aspergillus tamarri* KLD 2 were showed maximum zone of clearance. It showed around 0.45 cm zone of clearance observed.

Under natural conditions, depending upon various external factors, microflora varies from sample to sample. Therefore, the selection criteria, we have selected samples from different agricultural soils, rotten vegetables and wood-wastes in our initial screening approach.

Screenings of different substrates were used for the production of xylanase. The substrates were used are wheat bran, rice bran, wood chips, paddy husk, sugarcane waste and pigeon pea waste. In all the substrates maximum enzyme production were observed at 96th hr, the maximum production of xylanase were showed 5.77 IU in wood chips and lowest enzyme

production were observed in paddy husk and it showed 1.96 IU (Figure.3).

Okafor *et al.*, (2007) reported that the maximum production of xylanase were produced from *Aspergillus niger* ANL301 and it showed 6.47IU/mL by using wheat bran as a substrate among the other substrates such as Sawdust, Sugarcane pulps.

The large number of bacteria, yeast and filamentous fungi have been reported to produce Xylanase (Wong *et al.*, 1988), filamentous fungi are gaining importance as producers of xylanase over others from the industrial point of view due to non pathogenic nature, ease in cultivation under fermentation conditions and capable of producing high levels of extracellular enzymes (Kar *et al.*, 2006).

The maximum enzyme production is suggested by Svarachorn *et al.*, (2012) that the maximum xylanase obtained from *A. fumigates* strain 4-45-1F might be the sum of intracellular and extracellular xylanases. Maximum xylanase was obtained at the death phase when a mold pellet dispersion and lost in cell dry weight was observed. Our results were closely agree with the Okafor *et al.*, (2007).

References

- Ancharida Svarachorn. 1999. Production of Fungal-Xylanase using Agricultural Waste by Solid State Fermentation. *J. Sci. Res. Chula. Univ.* 24(1): 13-20.
- Collins. T., C.Gerday and Feller, G.2005. Xylanases, families and extremophilic xylanases. *FEMS Microbiol. Rev.* 29: 3-23
- Huisman. M.M.H., H.A. Schols and Voragem, A.G.I. 2000.

- Glucuronoarabinoxylan from maize kernel cell walls are more complex than those from Sorghum kernel cell walls. *Carbohydr. Polym.* 43: 269-279.
- Miller and Gail Lorenz. 1959. "Use of dinitrosalicylic acid reagent for determination of reducing sugar". *Anal. Chem.* 31 (3): 426-428.
- Okafor, U.A.L., V.L.L. Okochi, B.M. Onyegeme-okerental and Nwodo-Chinedu, S. 2007. Xylanase production by *Aspergillus niger* ANL 301 using agro – wastes African J. Biotechnol.6 (14): 1710-1714.
- Shallom, D., and Shoham, Y. 2003. Microbial hemicellulases. *Curr. Opin.Microbiol.* 6: 219-228.
- Wong, K.K.Y., L.U.L. Tan and Saddler, J.N. 1988. Multiplicity of α -1,4 xylanase in microorganisms' functions and applications. *Microbiol. Rev.* 52: 305-317.
- Wong, K.K.Y., L.U.L.Tan and Saddler, J. N. 1988. Multiplicity of 3-1,4-Xylanase in Microorganisms: Functions and Applications. *J. N. Microbiol. Rev.* 52(3): 305-317
- Yamaki, S., and Kakiuchi, N. 1979. Changes in hemicellulose-degrading enzymes during development and ripening of Japanese pear fruit. *Plant.Cell Physiol.* 20: 301-309.