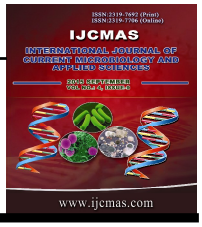
Int.J.Curr.Microbiol.App.Sci (2015) 4(9): 586-591

International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 4 Number 9 (2015) pp. 586-591**
http://www.ijcmas.com



Original Research Article

Isolation and Screening of Xylanase Producing Fungi from Forest Soils

G. Ramanjaneyulu, G. Praveen Kumar Reddy, K. Dileep Kumar and B. Rajasekhar Reddy*

Dept. of Microbiology, Sri Krishnadevaraya University, Anantapuramu, Andhra Pradesh, India
**Corresponding author*

A B S T R A C T

Fungi are well known for their ability to produce extracellular enzymes into their surrounding environment. Xylanases are important class of hydrolytic enzymes involved in degradation of xylan- a back bone of hemicellulose which is present in lignocellulosic biomass in considerable proportion into monomers. In this study, we have isolated 450 fungal cultures from forest soils of Eastern Ghats of Andhra Pradesh, India and screened by congored plate assay method followed by assessment of xylanase activity in submerged fermentation (SmF). All isolates exhibited hydrolytic activity of xylan in the form of zone of hydrolysis with in a range of 2.0 to 9.0 cm and only the cultures with greater than 5.0 cm of hydrolytic zone were further assessed for production of xylanase enzyme in SmF. Enzyme activity under SmF was measured by assessing the amount of reducing sugar released by using 3, 5 – Dinitrosalicylicacid (DNS) method.

Keywords

Congored plate assay, Fungi, Isolation, Screening, Xylanase

Introduction

Enzymes are distinct natural biological polymers which can catalyze the chemical/ biological reactions and convert substrates to specific product (Haq *et al.* 2006). Xylanase [E.C.3.2.1.8] are a class of hydrolytic enzymes which can hydrolyze the straight polysaccharide β-1, 4-xylan in hemicelluloses which is a noteworthy component of secondary cell wall of plants (Dhiman *et al.* 2008). Xylan, the most abundant in lignocellulosic feedstock in the form of hemicelluloses containing hetero polysaccharides, comprises of a backbone of β-1-4-linked xylopyranose residues with side chains of O-acetyl, arabinosyl and methyl glucoronsyl and forms an interface between lignin and other polysaccharides (Chang *et al.* 2003; Shanti *et al.*, 2014). Xylanases have gained a unique importance in the biotechnology and industries due to their potential applications. The major industrial applications of enzymes are in textile industry (Csiszár *et al.* 2001) for 'biopolishing' of fabrics and producing stonewashed look of denims, in household laundry detergents for improving fabric softness and brightness (Cavaco-Paulo, 1998), in food, leather, paper/pulp industries (Kirk and Jefferies, 1996; Bhat, 2000), in the fermentation of biomass for the biofuel production (Goldschmidt, 2008), in ruminant nutrition for improving

digestibility, in fruit juices processing and in de-inking of paper (Sakthivel *et al.* 2010).

Xylanases constitute one of the most important industrial enzymes that depolymerizes xylan molecule into xylose units (Garg *et al.* 2011).Microorganisms are rich sources of xylanase enzymes which are produced by diverse genera and species of fungi, bacteria and actinomycetes. Nonetheless, despite the fact that the vast diversity of fungal species known for secretion of xylanase, production of xylanase enzyme are largely harnessed from terrestrial isolates of *Aspergillus* and *Trichoderma* species in industrial scale (Fengxia *et al.*, 2008).However, search for organisms, in particular, filamentous fungi, with secretion of highest levels of xylanse enzymes are being continuously made for enrichment and diversity of xylanase-producing cultures in our stockpile (Polizeli *et al.*, 2005). In view of biotechnological and industrial applications, we have aimed our focus on isolation and screening of fungi for xylanase production.

Materials and Methods

Collection of soil samples

Soil samples were collected from different locations of forest in Rayalaseema region Andhra Pradesh within a depth of 6 to 12 cm after removal of superficial layer (approximately 0.5 mm). At each location 6 to 10 samples were collected individually within a range of 5 to 20 km distance and transported to laboratory and stored in a refrigerator at 4^{O} C until further study.

Culture media

For selective isolation of xylanase producing fungi Mineral Salts Medium (MSM) was prepared with NH_4NO_3: 1.5g, KH_2PO_4: 2.5g, NaCl: 1.0, $MgSO_4$: 1.5g, CaCl2: 0.05g, $MnSO_4$: 0.01g, $FeSO_4$: 0.005g in 1000 ml distilled water and were amended with xylose: 10.0 g/L or birchwood xylan 0.1%.pHof the medium was adjusted to 5.0 and sterilized at 121°C and 15 lb of pressure for 20 min and poured into sterile petri dishes.

Isolation of fungal colonies

Serial dilution technique was used for isolation of fungi from soils in which each sample of 1 g soil were suspended in 10mlof sterile distilled water and prepared stock and from this 1 ml of sample was serially transferred into 9 ml of sterile distilled water and prepared dilutions up to 10^{-8}dilution and plated on MSM-xylan/ xylose media over spreading of 100 µl of sample from 10^{-3} to $10^{-8}$for selective isolation of xylanase producing fungi.

Triplicates were maintained for each dilution. Single isolated colonies on plates were picked and further purified on fresh plates for axenic cultures and maintained on MSM- xylan/ xylose medium.

Screening of isolates for xylanase production

Initially, screening was performed by plate assay method in MSM-xylan medium by placingat center of the petriplate a culture disk of 0.5mm size plucked from 5-day old culture plateand incubated at 30 ^{O}C in an incubator (Technico) for 5 days.

After 5 days of incubation, the plates were flooded with 0.01% of 10-15 ml congored solution (Teather and wood 1982) and allowed to stand for 10 to 15 min and destained with 1% NaCl and the zone of hydrolysis was measured in cm.

Quantitative screening of microorganisms

In secondary screening, the cultures with zone of hydrolysis more than 5 cm were selected and further screened in submerged state fermentation (SmF) for the production of xylanase. Erlenmeyer flasks (250 ml) containing 30 ml of liquid MSM medium with 0.1% of birch wood xylan as carbon source was inoculated with 5 agar plugs of 0.5 mm size and incubated at 30°C and 150 rpm in an orbital shaker (Remi) for 7 days. The flasks were withdrawn at each day and filtered through Whatman No.1 filter paper. The filtrate was centrifuged at 8000g (Remi C - 24) at 4°C for 10 min and the supernatant was collected and used as enzyme source for the assay of xylanase activity.

Assay of xylanase

Xylanase activity was determined according to the method of Bailey *et al.,* (1992) by measuring the reducing sugar liberated from the xylan by 3, 5- dinitrosalicylic acid (DNS) (Miller 1959). Crude enzyme extract was taken as xylanase source and assayed in 3.0 ml of reaction mixture containing 1% birch wood (prepared in 0.05 M Na-citrate buffer, pH 5.3), 1 ml of 0.05 M citrate buffer and the reaction commenced with the addition of 1.0 ml of enzyme source and were incubated at 55°C for 10 min. The reaction was terminated by the addition of 3.0 ml of 3, 5- dinitrosalicylic acid (DNS) and the contents were boiled for 15 min in water both (Miller, 1959). After cooling, the color developed was read at 540 nm. The amount of xylose liberated was quantified using xylose as standard. One unit of xylanase activity (U) is defined as the amount of enzyme that liberates 1 µmol of reducing sugar - xylose per min under the standard assay conditions.

Results and Discussion

Fungi play a vital role in degradation of lignocellulose polymer by the secretion of necessary enzymes which are involved in the depolymerization of lignocellulose. In the present study, results showed that all fungal isolates showed xylanolytic activities as detected by the solid medium assay followed by SmF fermentation.

Primary screening

Plate assay with congored is one of the important methods to assess the xylanolytic activity of the microflora. This method was initially developed by Teather and Wood (1982) and it is depends on the formation of complex between the dye congored and polysaccharide (xylan) the congored (reactive dye). A clear halo zone will appear after addition of dye and destaining with NaCl on plates due to the disappearance of polysaccharide around growing colony because of utilization of polysaccharide by mocroorganisms with secretion of xylanase. The screening of fungal cultures for xylanolytic activity on plate method showed the clear zone after de-staining with in a range of 0.5 to 11 cm (Figure 1).

The cultures, showing zone of clearance with diameter greater than 5.0 cm were further tested for the production of xylanase in SmF. Fungal cultures isolated through two methods (xylan – and xylose – containing medium) and their zone of clearance on xylancongo red plate are presented in (Table 1 & 2).

Among fungal isolates, all isolates showed xylanolytic potential as examined based on Congo red staining (Figure 1). The halo zones around the fungal colonies signified the solubilisation of xylan due to the hydrolytic action of xylanases.

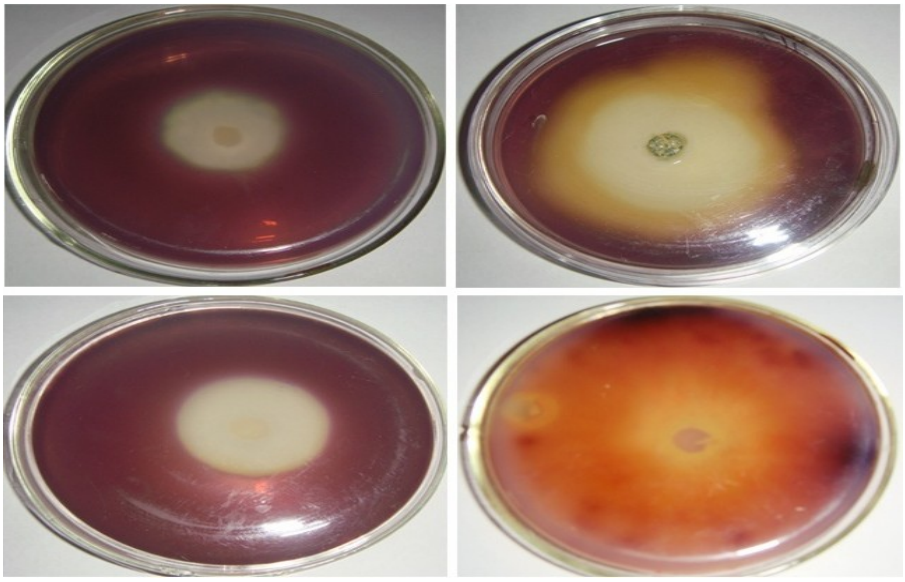
Table.1 Zone of clearance by fungal cultures isolated through xylan- MSM

Culture	Zone of clearance in cm	Culture	Zone of clearance in cm
D 10 a	6.8	**I 1**	5.4
Q4	8.6	**B 21**	6.4
N 7	8.0	**L 9**	6.8
D 19	5.3	**L 8**	5.8
J 5	5.5	**J 3**	6.6
C 3	6.0	**B 24**	5.4
M 4	6.0	**L 1**	6.2
B 18	5.5	**M 1**	5.5

Table.2 Zone of clearance of fungal cultures isolated through xylose- MSM

Culture	Zone of clearance in cm	Culture	Zone of clearance in cm
B 41	9.0	**A3**	6.3
F 3	5.5	**L 1**	9.1
Q 24	5.3	**D 1**	5.3
H 5	8.7	**H 7**	7.0
D 20	8.5	**C 20**	5.5
G 2	6.4	**I 10**	6.2
K 2	6.0	**Q 22**	5.5
Q 12	5.5		

Figure.1 The zone of hydrolysis produced by bacterial strains on nutrient agar medium containing xylan



Nature (forest soil) is considered as the wealthiest and the most diverse hotspot for an assortment of microorganisms which produce never-ending of enzymes critical for applications in different industrial processes. Martin *et al.,* (2013) screened fungi of the mangrove forest (MF) for the xylanase production on the solid medium assay. Eight positively screened MF isolates (*Aspergillus* sp. PAQ-H, *Aureobasidium* sp. 2LIPA-M, *Colletotrichum* sp. WABA-L, *Fusarium* sp. KAWIT-A, *Paecilomyces* sp. FDCAB-7, *Guignardia* sp. 2SANQ-F, *Penicillium* sp. LIABA-L, and *Phomopsis* sp. MACA-J) were selected for xylanase production in liquid medium. *Aspergillus candidus* was isolated from soil sample collected from Meerut Institute of Engineering and Technology, Uttar Pradesh (Garai and Kumar, 2013)

Secondary screening

The degree of clear zone formation in the screening in previous experiments demonstrated the ability of fungi to utilize xylan as a carbon source by the secretion of an enzyme xylanase. Fungal cultures, selected on the basis of zone of hydrolysis, were grown on xylan-MSM in submerged fermentation. In SmF all selected isolates (made through the first method) produced xylanase with in a range of 50 to 1250 U/ml whereas the selected isolates made through the second method yielded titers of 320 to 1290 U/ml. Senthilkumar *et al.* (2005), Abdullah *et al.* (2014), Saha *et al.* (2014), Bekkarevich *et al.* (2015), Adhyaru *et al.* (2015) and Pereira *et al.* (2015) reported xylanase activity of fungal and bacterial cultures within a range of 182 to 3060 U and by following the same method employed in the present study (Biely *et al.*, 1992).

Forest soil samples are a massive treasure-house of xylan- and xylose-utilizing microflora for exploration. Some of the fungal cultures derived from this study exhibited xylanase activity as high as 4560 U/ ml after optimization.

References

Abdullah R, Nisar K, Aslam A, Iqtedar M, Naz S. 2014. Enhanced production of xylanase from locally isolated fungal strain using agro-industrial residues under solid-state fermentation, *Natural Product Research (Formerly Natural Product Letters)*, 29(11): 1006-1011.

Adhyaru DN, Bhatt NS, Modi HA. 2015. Optimization of upstream and downstream process parameters for cellulase-poor-thermosolvent-stable xylanase production and extraction by *Aspergillus tubingensis* FDHN1. *Bioresource Bioprocessing.*, 2(3): 1–14.

Bailey M, Biely J, Poutanen K. 1992. Interlaboratory testing of methods for assay of xylanase activity. *Journal of Biotechnology.*,23(3): 257-270.

Bekkarevich AO, Nemashkalov VA, Koshelev AV, Goryachev DA, Bubnova TV, Matys VY, Osipov DO, Kondrat'eva EG, Okunev ON, Sinitsyn AP. 2015. Cultivation of a novel cellulase/ xylanase producer, *Trichoderma longibrachiatum* Mutant TW-1-59-27: 13 Production of the enzyme preparation and the study of its properties. *Appl Biochem Microbiol.*, 5(2): 229-235.

Bhat MK.2000.Cellulases and related enzymes in biotechnology. *Biotechnol Adv.*,18:355-383.

Cavaco-Paulo A. 1998. Mechanism of cellulase action in textile processes. *Carbohydrate Polymers*., 37:273- 277.

Chang A. 2003.Schornburgdietmar and SchomburgIda. Springer Handbook of Enzyme, Springer,

Csiszár E, Urbánszki K and Szakás G. 2001.Biotreatment of desized cotton fabric by commercial cellulase and xylanase enzymes.*J MolCatal B Enzym.*,

11:1065-1072.

Dhiman S S, Sharma J and Battan B. 2008. Industrial Applications and future prospects of Microbial Xylanases: A Review. *BioResources.*, 3(4): 1377-1402.

Fengxia L, Mei L, Zhaoxin L, Xiaomei B, Haizhen Z, Yi W.2008. Purification and characterization of xylanase from *Aspergillus ficuum* AF-98.*Bioresour Technol*., 99: 5938–5941.

Garai Debabrataand Kumar. 2013.Response surface optimization for xylanase with high volumetric productivity by indigenous alkali tolerant *Aspergillus candidus* under submerged cultivation.*3 Biotech*., 3(2): 127–136.

Garg G, Dhiman SS, Mahajan R, Kaur A and Sharma J. 2011.Bleach-boosting effect of crude xylanase from *Bacillus stearothermophilus* SDX on wheat straw pulp.*New Biotechnol*., 28(1):58-64.

Goldschmidt F. 2008. From Cellulose to Ethanol: Engineering Microorganisms to Produce Biofuel. Institute of Biogeochemistry and Pollutant Dynamics 1-17.

Haq IU, Javed MH and TM Khan.2006. An innovative approach for hyperproduction of cellulolytic and hemicellulolytic enzymes by consortium of *Aspergillusniger*MSK-7 and Trichoderma viride MSK-10. *Afr J Biotechnol.*, 5(8):609-614.

Kirk TK, Jefferies TW. 1996. Role of microbial enzymes in pulp and paper processing. In: Jefferies TW, Viikari L (*eds*)Enzymes for pulp and paper processing. ACS symposium series. American Chemical Society, Washington, 1-14.

Martin Jeremy O, Torres, Thomas Edison E, dela Cruz.2013. Production of xylanases by mangrove fungi from the Philippines and their application in enzymatic pretreatment of recycled paper pulps. *World J MicrobiolBiotechnol.*,29: 645–655.

Miller GL. 1959. Use of 3, 5- dinitrosalicylic acid reagent for determination of reducing sugar.*Anal Chem.*, 31: 426-428.

Pereira JC, Marques NP, Rodrigues A, Oliveira BT, Boscolo M, Da Silva R, Gomes E, Martins DAB. 2015. Thermophilic fungi as new sources for production of cellulases and xylanases with potential use in sugarcane bagasse saccharification. *J Appl Microbiol.*, 118(4): 928-939.

Polizeli MLTM, Rizzatti ACS, Monti R, Terenzi HF, Jorge JA, Amorim DS.2005. Xylanases from fungi: properties and industrial applications. *ApplMicrobiolBiotechnol.*,67: 577–591.

Saha SP, Ghosh S. 2014. Optimization of xylanase production by *Penicilliumcitrinum*xym2 and application in saccharification of agro-residues.*Biocatalysis and AgriBiotech.*, 3: 188-196.

Sakthivel M, Karthikeyan N, Jayaveny R, Palani P. 2010. Optimization of culture conditions for the production of extracellular cellulase from *Corynebacterium lipophiloflavum*. *J Ecobiotechnol.*, 2(9):06-13.

Senthilkumar SR, Ashok Kumar B, Chandra Raj K, Gunasekaran P. 2005. Optimization of medium composition for alkali- stable xylanase production by *Aspergillusfischeri* Fxn1in solid-state fermentation using central composite rotary design.*Bioresource Tech.*, 96: 1380-1386.

Shanthi V, andRoymon MG. 2014. Isolation and Screening of Alkaline Thermostable Xylanase Producing Bacteria from Soil in BhilaiDurg Region of Chhattisgarh, India. *Int.J.Curr.Microbiol.Appl.Sci.*, 3(8): 303-311.

Teather RM, Wood PJ. 1982. Use of congo red-polysaccharide interactions in enumeration and characterization of cellulolytic bacteria from the bovine rumen. *ApplEnvMicrobiol.*, 43 (4): 777-780.