International Journal of Advanced Research in Biological Sciences
ISSN: 2348-8069
www.ijarbs.com
DOI: 10.22192/ijarbs Coden: IJARQG(USA) Volume 4, Issue 3 - 2017

Research Article

DOI: http://dx.doi.org/10.22192/ijarbs.2017.04.03.011

Isolation and identification of xylanase producing fungal isolate

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Abstract

Fungal isolate SATU 1 producing xylanase was isolated from decaying wood and soil sample collected from Tumkur region. The fungal isolate was identified by microscopic and other conventional methods. The selected potent fungal isolate SATU 1 was screened on Czepox dox agar containing (1% xylan) as a substrate. Isolate SATU 1 showed maximum zone of 46 mm on screening media. SATU1 showed maximum enzyme activity of 14.24 IU/ml. Xylanase produced from the isolate was comparable with other published data.

Keywords: Xylanase, Fungi, Screening, Identification, Production

Introduction

Xylans are hemicelluloses and the second most abundant natural polysaccharide (Collins *et al.* 2005). Xylan is a complex heteropolysaccharide having a highly branched chain of 1, 4-linked xylanopyranosyl residues (Bastawade 1992). Xylanases (EC 3.2.1.8) catalyze the hydrolysis of xylan, the major constituent of hemicelluloses found in plant cell wall. (Kulkarni *et al.*, 1999). Xylanases are produced by diverse group of organisms such as bacteria, algae, fungi, protozoa, gastropods and arthropods (Collins *et al.*, 2005).

Fungi are the major producers of biomass degrading enzymes such as cellulases and hemicellulases in industry. Fungi are of special interest as they secrete enzymes in large quantities. Xylanase titres are much higher than those found in yeast and bacteria (Haltrich *et al*. 1996). Xylanases show great potential for industrial applications in the bioconversion of lignocelluloses to sugar, ethanol, and other useful substances, clarification of juices and wines, improving the nutritional quality of silage and green feed and the de-inking processes of waste papers (Viikari *et al.*, 2001). Interest in xylanolytic enzymes has increased due to their potential use in several industrial processes such as biopulping and bleaching; bioconversion of lignocellulose; food processing, including clarification of beer, wine, and juice; increasing digestibility of animal feedstock; and bread making (Bastawade 1992 : Vikari *et al* 1994,Uffen 1997).

Materials and Methods

The chemicals used in the present study were of analytical grade. Decayed wood sample, Soil and garden samples were collected in a sterile polythene

cover from different areas of Tumkur Township. The sample was processed carefully. Fungi were preliminarily isolated by dilution plate method on Czapek Dox Agar medium. Pure cultures of individual isolates were identified by conventional methods (Gilman, 1959; Ellis, 1971 and Barnett *et al* 1972). All fungal isolates were screened for their abilities to produce extracellular xylanase during their growth on Czapek's agar medium containing xylan as the sole carbon source. The composition of the medium was (g.L-1): Xylan (Hi media), 0.1%; peptone, 5.0; yeast extract, 5.0; K_2HPO_4, 1.0; $MgSO^4.7H_2O$, 0.2 and agar, 20.0. The inoculated plates were incubated for 7 days at 30ºC. Plates were flooded with 0.1% (w/v) Congo red after 30 min, washed with 1 M NaCl and observed for zone of clearance around the fungal growth. Fungal strains, which produced distinct clearing zones around their colonies, were selected for further studies.

Identification of the fungal isolate

Fungal identification was done based on structural characters and colony morphology observed under light microscope**.** Colour, texture and pigmentation of the isolates were recorded. Mount prepared was stained with lacto phenol cotton blue. The fungal characteristics were described and identified based on the description given by Pitt and Samson (2000).

DNA extraction and 18S rRNA identification

The fungal DNA was isolated and purity of DNA was quantified by running it on Agarose gel electrophoresis. 18s rRNA gene primers were used to amplify ~1.1Kb gene from the genomic DNA. PCR product was quantified after purification sent for sequencing. Sequences obtained was analysed and aligned. Aligned Sequences subjected to BLAST with NCBI Genbank database. Based on maximum identity score the fungal isolate was identified.

Production of xylanase

The fungal isolate which showed maximum zone consistently on the screening media was selected for the production of xylanase. The spore suspension was prepared by adding spore suspension of fungal growth from the 7 day incubated SDA plate in to 50 ml of sterile distilled water. The tube was shaken to make homogeneous mixture of spore suspension. The cultivation was carried out in a Czapek Dox liquid medium containing (1%) xylan as the sole carbon source. An Erlenmeyer flask (250 ml) containing 100 ml of the growth medium was inoculated with $5X10^8$ spore suspension and cultured for 15 days at 28 °C on an orbital shaker set at 250 rev min-1.Broth was filtered with Whatsman filter paper . The Filterate was centrifuged at 10,000 rpm for 15 min at 4^0C. The culture supernatant obtained was used for the assay of xylanase activity.

Estimation of xylanase activity

Xylanase activity was estimated as per the method of Miller (1959)

Reaction mixture contains 0.9 ml of (1%) Brich wood xylan dissolved in 50mM Buffer of pH 8.6 the prepared substrate was pre incubated at 50˚ C for 15 minutes. To the above test tube 0.1 ml of enzyme was mixed with 0.1ml of buffer. The above mixture was incubated at 50˚ C for 15 minutes. The reaction was terminated by adding 1.5 ml of 1% (w/v) DNS. Colour developed was measured colorimetrically. Enzyme activity was expressed as the enzyme required to release one micromole of the reducing sugar equivalent to one micromole of xylose per minute at standard assay condition.

Results and Discussion

In the present study the Xylanase producing fungal isolate was isolated from decayed wood sample. The selected isolate SATU1 showed prominent zone on the Czepox Dox Agar screening media as observed in Fig: 1.1. The potent isolate was identified as *Aspergillus niger* based on its growth; colour and pigmentation .*Aspergillus* is widely distributed geographically, and also observed in different type of habitats. The growth of *Aspergillus* depends on the influence of temperature, water activity and pH. Filamentous fungi have better growth at low water activities compared to yeasts and bacteria (Bhardwaj *et al.,* 2011). Xylanases produced from the fungal strain *Trichoderma* sp. and *Aspergillus* sp have widely studied and employed in different industries such as bakery and food processing industries (Wong and Saddler, 1992; Kulkarni *et al.,* 1999).The fungal isolate SATU1 showed the zone of 46 mm on screening media. The 18s rRNA gene sequencing is the most reliable method for the identification of fungal isolates. The Blast result of the SATU1 with other fungal isolates sequences showed similarity of 90 to 99% with *Aspergillus nigeer.* Optimization of physicochemical parameter is must for the enhanced production of the desired enzyme. The selected potent isolate produced Xylanase of 14.24 IU/ml, which is comparable with the work done by other researchers. The chemicals and the wastes released from pulp and paper industry have a posed a

threat to the environment. The use of enzyme alternate to harsh chemical reduced the pollution to the greater extent. Xylanases are used in the prebleaching of kraft pulp to reduce the use of harsh chemicals in the subsequent chemical bleaching stages. The enzymatic treatments improve the chemical liberation of lignin by hydrolyzing residual xylan. This reduces the need for chlorine-based bleaching chemicals, which is beneficial for the environment. (Beg *et al*., 2001).Use of Xylanase in pulp industry reduced the content of hexenuronic acids (HexA) used in pulp industry. (Jiang *et al.*, 2006; Sevastyanova *et al.*, 2006).The xylanase producing fungal isolate SATU 1 showed promising results. Further optimization of enzyme will enhance the enzyme production.

Table 1.1 Comparative evaluation Xylanase producing fungi

S. No	Author	Microorganism	Zone of clearance	Enzyme activity
1	Padmavati & Kavya 2011	*Aspergillus niger*	ND	16 U/ml
2	Chandra et al 2012	*Aspergillus Niger*	60 mm	ND
3	Adesina and Onllude 2013	*Aspergillus niger* ANMA	15mm	4.29 U/ml
4	Dhulappa and lingappa 2013	*Aspergillus tammari* KLD 2	0.45cm	5.77 IU
5	The present study	*Aspergillus*	46mm	14.24 U/ml

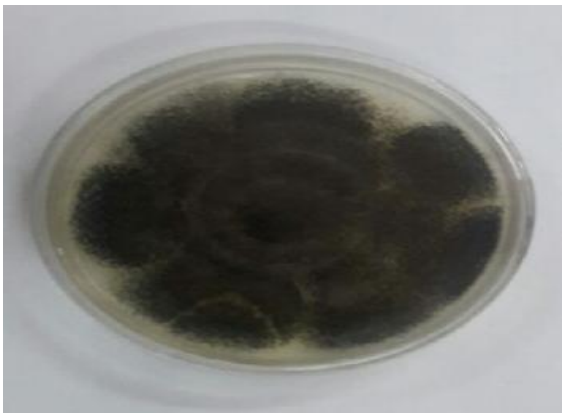

Fig 1.1 Plate photo of *Aspergillus niger*

5’TTGGATGGTGGTGCATGGCCGTTCTTAGTTGGTGGAGTGATTTGTCTGCTTAATTGCGATAACGAACG
AGACCTCGGCCCTTAAATAGCCCGGTCCGCATTTGCGGGCCGCTGGCTTCTTAGGGGGACTATCGGCTC
AAGCCGATGGAAGTGCGCGGCAATAACAGGTCTGTGATGCCCTTAGATGTTCTGGGCCGCACGCGCGCT
ACACTGACAGGCCAGCGAGTACATCACCTTGGCCGAGAGGTCTGGGTAATCTTGTTAAACCCTGTCGTG
CTGGGGATAGAGCAGCAATTATTGCTCTTCAACGAGGAATGCCTAGTAGGCACGAGTCATCAGCTCGTG
CCGATTACGTCCCTGCCCTTTGTACACACCGCCCGTCGCTACTACCGATTGAATGGCTCGGTGAGGCCTT
CGGACTGGCTCAGGAGGGTTGGCAACGACCCCCCAGAGCCGGAAAGTTGGTCAAACCCGGTCATTTAG
AGGAAGTAAAAGTCGTAACAAGGTTTCCGTAGGTGAACCTGCGGAAGGATCATTACCGAGTGCGGGTC
CTTTGGGCCCAACCTCGCATCCGTGTCTATTGTACCCTGTTGCTTCGGCGGGCCCGCCGCTTGTCGGCCG
CCGGGGGGGGCGCCTCTGCCCCCCGGGCCCGTGCCCGCCGGAGACCCCAACACGGGGGGAACTGCGGA
AGGATCATTACCGAGTGCGGGTCCTTTGGGCCCAACCTCCCATCCGTGTCTATTGTACCCTGTTG’3

Fig 1.2 Sequences *Aspergilus niger*

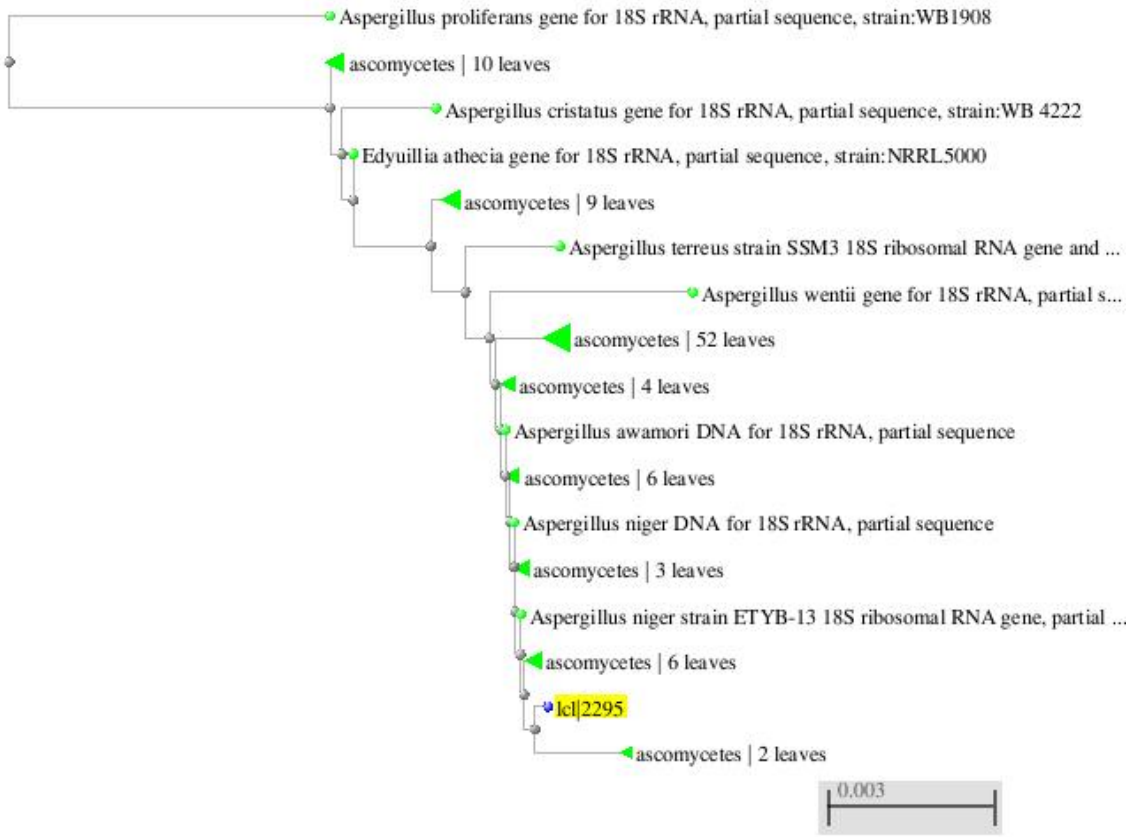

Fig 1.3 Phylogenetic tree *Aspergilus niger*

Conclusion

From the present study it can be concluded that a potent Xylanase producing fungal isolate SATU1 was isolated from decayed wood sample and screened on Czepox Dox Agar media containing (1% birch wood Xylan) as a substrate. Fungal isolate was identified as *Aspergillus niger* based on conventional and 18S r Rna sequencing method. It showed zone of 46mm on screening media. Potent isolate produced 14.24 IU/ml of enzyme. The exploration of the biodiversity and exploitation of microbial enzyme is good for the humanwelfare and for the environment. The overall observation highlights the potentiality of Xylanase producing fungal isolate *Aspergillus niger*.

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DOI:10.22192/ijarbs.2017.04.03.011	

How to cite this article:
Shabeena Anjum K S, Ravi M, Y M Jayaraj. (2017). Isolation and identification of xylanase producing fungal isolate. Int. J. Adv. Res. Biol. Sci. 4(3): 102-106.
DOI: http://dx.doi.org/10.22192/ijarbs.2017.04.03.011