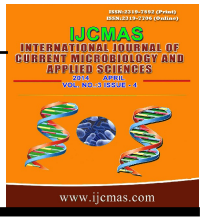
International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 3 Number 4 (2014) pp. 501-506**
http://www.ijcmas.com



Original Research Article

A reviving preliminary evoke on few xylanase producing fungal isolates from different ecological niche

Vishal Kumar, Pragya Pandey, Shivani Gupta, Pratyoosh Shukla*

[1]Enzyme Technology and Protein Bioinformatics Laboratory, Department of Microbiology, Maharshi Dayanand University, Rohtak-124001, Haryana, India
Corresponding author

Keywords

Fungi;
YPSS media;
Xylanase,
Thermomyces lanuginosus;
Aspergillus sp.I.

A B S T R A C T

In the present pre-screening report we describe the occurrence of few xylanase producing fungi from different ecological niche i.e. soil samples and plants debris samples. In the present work, diverse soil samples were collected from these sites and fungal isolates were grown on YPSS media. Among five isolates three different fungal isolates as identified morphologically through colonial morphology and adapting basic microbiological procedures were TMDU1 (*Thermomyces lanuginosus*), TMDU2 (*Aspergillus* sp.I) and TMDU3 (*Aspergillus* sp.II). The qualitative xylanase assay was carried out which revealed that all the selected isolates are xylanase producer. Further, TMDU1 (*Thermomyces lanuginosus* spp.) was selected for further studies owing to its better xylanase producing capability. Further, few studies on physiological profiles of these isolates suggested xylan and yeast extract as best carbon and nitrogen source respectively..

Introduction

Lignocellulose is the chief component of the overall ecological biomass it is composed of major biological macropolymers such as cellulose, hemicelluloses and lignin. Hemicelluloses are complex mixtures of different polymers such as xylan, mannan, galactan, arabinan or other heteropolymers (Verma and Satyanarayana, 2012). Xylan is a major polysaccharide which is composed of xylose units linked by β -1,4 glycosidic linkage, a mixture of hydrolytic enzymes is required to complete degradation of xylan, among them xylanase plays an important role (Takahashi et al 2013). Xylanase is an industrially important enzyme which degrades xylan randomly by its endo-1,4-β-xylanase activity and produces xylose, xylooligosaccharides and xylobiose (Sharma and Kumar, 2013). Xylanases have been found applications in paper and pulp industries and in the production of bio-ethanol etc (Bhat and Hazlewood, 2001). For industrial applications, xylanases must be optimally active in alkaline range and high temperatures (Shrivastava et al 2011).

There are several groups of microorganisms that are very rich sources of thermostable xylanases, such as bacteria, actinomycetes, and fungi (Suneetha, 2011; Kamble and Jadhav, 2012). Among these groups several genus of filamentous fungi secrete high amounts of extracellular thermophilic xylanases. There is some important genus of filamentous fungi, which produces xylanases such as *Thermomyces, Trichoderma, Aspergillus* etc. (Takahashi et al 2013; Shrivastava et al 2013). *Themomyces lanuginosus* (previously known as *Humicola lanuginose*) is a thermophilic fungus widely distributed in self-heating mass of organic debris and soil (Singh et al 2003). This fungus produces thermostable and alkalistable xylanases which is best suitable for bio-bleaching process in paper and pulp industry. In the present work, we are reporting a swift description on few xylanase producing fungal isolates from different ecological niche.

Materials and Methods

Sample, media and growth conditions

The different soil samples and plants debris samples were collected from the pot and garden of M.D. University, Rohtak. Soil suspensions in sterilized distilled water (0.1 g/ml) were prepared and serially diluted from 10^{-1} to 10^{-3} dilutions and 100 µl of each diluted sample were spread onto agar plates (YPSS: Yeast extract- 4 g/l, Soluble Starch- 15 g/l , $MgSO_4$- 1 g/ l, K_2HPO_4- 1 g/l, Agar- 20 g/ l ; PDA: Potato infusion- 200 g/l, Dextrose 20 g/ l, Agar 20 g/ l; SDA: Dextrose 40 g/l, Peptone 10 g/l , Agar 20 g/l) containing 0.8 g/l Kanamycin. The plates were incubated at 50^{o}C for 3-4 days. Fungal colonies were isolated and maintained on YPSS slants and stored at 4°C. The slant cultures were sub cultured every month.

Primary screening (Zone of clearance)

Fungal isolates were screened for xylanase production on Czapek's agar medium (Birch wood Xylan- 5 g/l, Peptone- 5 g/ l, K_2HPO_4- 1 g/l, $MgSO_4$ - 1 g/l**,** Agar 20 g/l). After inoculation the plates were kept at 50°C for 6 days and then the plate was treated with Congo red and washed with 1 M NaCl to observe the solubilisation zone (Tallapragada and Venkatesh, 2011). The confirmed fungal isolates were found to be xylanase positive and some selected isolates were maintained on YPSS agar plates for further studies.

Qualitative Enzyme Assay

Spore Suspension

Slants were prepared in Erlenmeyer conical flasks each of 250 ml containing 50 ml of YPSS agar medium. Each fungal isolates were inoculated in respective flask and incubated at 50°C for 7 days. A saline solution in 150 ml distilled water by adding 0.15 ml Tween-80 (0.01%) and 1.23 g NaCl was prepared. A quantity of 50 ml of this solution was added in each flask and mycelium was scratched with a sterilized loop and filtered with autoclaved filter assembly.

Solid State Fermentation (SSF)

Solid State Fermentation for xylanase production was carried out in Erlenmeyer flasks (250 ml) containing 10 g of wheat bran as substrate which was moisturised with 10 ml of distilled water. These flasks were sterilized and were inoculated with 1 ml of spore suspension at concentration

10^5 -10^6/ml and the flasks were incubated at 50°C in stationary condition. The samples were obtained after 72 hrs and were filtered using filter paper (Whatman no. 1). Further, the clear supernatant was collected for xylanase assay.

Xylanase Assay

Xylanase activity was determined by measuring the amount of reducing sugars liberated from birchwood xylan used as a substrate. Dinitrosalicylic acid (DNS) method was used to determine reducing sugar concentration (Shrivastava, et al. 2011). The substrate was prepared by dissolving birchwood xylan in acetate buffer pH 5.0 (1.0% w/v). The reaction mixture containing 1 ml of substrate solution and 1 ml of enzyme solution (crude enzyme) and incubated for 30 min at 50ºC, then the reaction was stopped by adding 3 ml of DNS reagent and reading were taken by spectrophotometer at 540 nm.

Results and Discussion

Isolation and primary Screening

A total of five fungal strains namely TMDU1, TMDU2, TMDU3, TMDU4 and TMDU5 were isolated from the soil and plant debris sample. All of these isolates were showing optimum growth at 50°C. All of these thermophilic fungal isolates were screened for xylanase production on Czapek Dox Agar medium. It was observed that only three fungal isolates TMDU1, TMDU2 and TMDU3 were able to grow on this medium. The results of primary screening (zone of clearance) presented that TMDU1 showing maximum xylanase production as compared to other two isolates viz. TMDU2 and TMDU3.

Morphological and microscopic characters

The isolates TMDU1 showing gradual colour change during its growth due to pigment production. Initially at second day it shows white filamentous growth, which gradually turned light yellowish to light brown it showed brown colour at fourth day and finally, at sixth day it was wine coloured on YPSS medium. During microscopic examination of TMDU1 it was observed small conidia were attached to hyphae all over its length and free conidia which get detached from hyphea were randomly scattered as visible under light microscope. Whereas TMDU2 and TMDU3 were black and brownish yellow in colour and there were no significant colour change were observed. It was studied under microscope TMDU2 have large globose conidia and TMDU3 have comparatively small columner conidia attached to terminal end of hyphae.

Xylanase estimation

All the three isolates were producing colour during xylanase assay with DNS the isolate TMDU1 was showing highest colour intensity (OD 0.684) so this isolate was recognised as best xylanase producer. This isolate was confirmed as *Thermomyces sp.* by morphological characteristics and microscopic conidial and hyphae structures characteristics.

A total of five thermophilic fungal isolates were recorded from different ecological niche such as potted plant, soil from garden, plant debris from garden. Three isolates (TMDU1, TMDU2 and TMDU3) were found xylanase producers; this reflects that a high percentage (60%) of xylanase producers is prevalent in these samples. Further these isolates were

Figure I *Thermomyces lanuginosus* TMDU1 (96 Hrs old culture)

Figure II *Thermomyces lanuginosus* TMDU1 (40X) showing hyphae and condidia

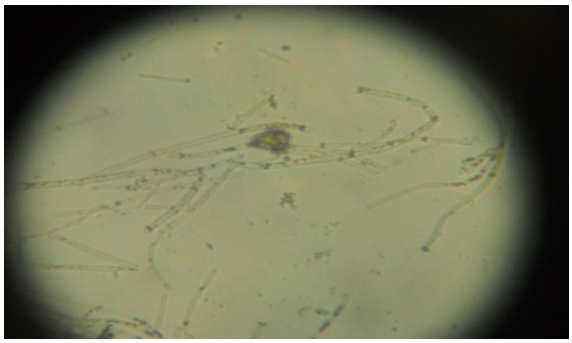


Table.1 Morphological and microscopic characteristics of different fungal isolates and xylanase producing capability

Isolate No.	Colony characteristics	**Microscopic identification**	Isolate Identification	**Xylanase producing capability/Zone of clearance**
TMDU1	Regular change in colour pale white to dark brown, mycelial growth spreaded on whole plate	Small conidia were attached to hyphae all over the its length and some conidia get deattached from hyphae spreaded randomly	*Thermomyces sp.*	+++
TMDU2	Black coloured, not significant change in colour, mycelia mat with regular margin	Large globose conidia attached at the tip of hyphae	*Aspergillus sp.I*	++
TMDU3	Brownish yellow coloured, slight colour change yellow to brownish yellow, regular shaped margin	Small columner conidia attached at the tip of hyphae	*Aspergillus sp.II*	+
TMDU4	White coloured mat have oval shape with regular margin	Not identified	Not identified	nd
TMDU5	Green coloured powdery growth with, no colour change , irregular margin	Not identified	Not identified	nd

Abbreviations:+++: Excellent; ++: Good; +: Fair nd: Not detected

identified on the basis of their morphological and microscopic characteristics which revealed them as *Thermomyces sp.* (TMDU1), *Aspergillus sp.I* (TMDU2), *Aspergillus sp II* (TMDU3). A review of literature suggests that there are related studies were reported by Takahashi et al (2013) and Shrivastava et al. (2013). Although there are several other reports on xylanase producing fungal species such as *Penicillium sp.*, *Chaetomium sp.*, *Fusarium sp.* etc. (Sharma and Kumar, 2013) but *Thermomyces sp.* is reported as one of the best xynalase producers by many researchers, The reports of xylanase production from *Thermomyces lanuginosus* IOC-4145 (Monica et al 2003), *Thermomyces lanuginosus* DSM 5826 (Khucharoenphaisana et al 2008) and *Thermomyces lanuginosus* SS8 (Shrivastava et al 2011) are noteworthy in this perception. It is also envisaged that *Thermomyces lanuginosus* xylanases are found to be thermostable and pH tolerant so they can be used as most excellent appropriate choice for the pulp and paper industry. The further purification and bioprocess optimization for such xylanases at lab scale and pilot scale are under progress that may interpret fascinating results.

Lignocellulolytic enzymes are widely used in various industries e.g. pulp and paper, bio-ethanol, food industry etc. and xylanases from *Thermomyces lanuginosus* is a quite important in this context. The efforts could be made to optimize and set a cost effective bio-process and prove its applicability as a viable option for these industries. The evolution of novel xylanase function requires information on protein structure, sequence along with phylogeny. Overall, these may help in predicting the newer function and application of xylanases as well as in designing new enzymes for their use in above-mentioned industries.

Acknowledgment

The authors duly acknowledge the financial support from SERB, Department of Science and Technology, Govt. of India (DST Fast Track Grant. No. SR/FT/LS-31/2012).

References

Bhat, M.K., and Hazlewood, G.P. 2001.Enzymology and other characteristics of cellulases and xylanases. In: Bedford MR, Partridge CC (eds) Enzymes in farm animal nutrition. CAB International,Wallingford., pp. 11–60.

Kamble, R. D., and Jadhav, A.R.2012.Isolation, Purification, and Characterization of Xylanase Produced by a New Species of *Bacillus* in Solid State Fermentation. International Journal of Microbiology. http://dx.doi.org/10.1155/2012/683193 .

Khucharoenphaisana, K., S. Tokuyamab and Kitpreechavanicha, V. 2008. Characterization of the thermostability of xylanase produced by new isolates of *Thermomyces lanuginosus*. ScienceAsia 34: 187–192.

Monica C., T. Damaso, M.S. Almeida, and Albano R.M. 2003. Optimized Expression of a Thermostable Xylanase from*Thermomyces lanuginosus* in *Pichia pastoris*. Applied and Environmental Microbiology 69(10): 6064-6072.

Sharma, M., and Kumar, A.2013.Xylanases: An Overview. British Biotechnology Journal. 3(1): 1-28.

Shrivastava, S., P. Shukla and Mukhopadhyay, K. 2011. Purification and preliminary characterization of a xylanase from *Thermomyces lanuginosus* strain SS-8. 3 Biotech. 1(4): 255-259.

Shrivastava, S., P. Shukla, P.D. Deepalakshmi and Mukhopadhyay, K. 2013. Characterization, cloning and functional expression of novel xylanase from *Thermomyces lanuginosus* SS-8 isolated from self-heating plant wreckage material. World Journal Microbiology and Biotechnology. (12): 2407-2415.

Singh, S., A.M. Madlala and Prior, B. A. 2003. *Thermomyces lanuginosus*: properties of strains and their hemicellulases. FEMS Microbiology Reviews. 27: 3-16.

Suneetha ,V., 2011. Actinomycetes : Sources for Soil Enzymes Soil Enzymology, Soil Biology, edited by G Shukla and A Varma (Springer-Verlag Berlin, Heidelberg). pp. 259.

Takahashi,Y., H. Kawabata and Murakami, S. 2013. Analysis of functional xylanases in xylan degradation by *Aspergillus niger* E-1 and characterization of the GH family 10 xylanase XynVII. SpringerPlus. 2: 447.

Tallapragada, P., and Venkatesh, K.2011.Isolation, identification and optimization of xylanase enzyme produced by *Aspergillus niger* under submerged fermentation. Journal of Microbiology and Biotechnology ResearcH. 1(4): 137-147.

Verma, D., and Satyanarayana, T.2012.Molecular approaches for ameliorating microbial xylanases. Bioresource Technology.17: 360–367.