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Finding Potential Source of Cold-Active Xylanase

S. M. Malik[1*], F. A. Ahanger[2], N. Wani[3], S. Sahay[4], K. Jain[5]

[1,2,3,5]Govt. Science and Commerce College Benazeer, Bhopal -462008 (MP) India
[4]Department of Botany, NSCB Govt. PG College, Biaora, Rajgarh (MP) India

**Corresponding Author: suhaibmlk07@gmail.com, Mobile: +919906500200*

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Abstract- Xylanases are glycosidases that catalyze the endohydrolysis of 1,4-β-D-xylosidic linkages in xylan, the main constituent of hemicelluloses found in plant cell wall. Xylanases encompass great industrial potential, and their cold-active counterparts have even more than that. Accordingly, three cold-active fungi, *Penicillium canesence* (BPF4), *Truncatella angustata* (BPF5), and *Pseudogymnoascus roseus* (BPF6) available as laboratory stocks have been screened for their ability to produce extracellular xylanases at cold temperature. The selection of hyper producing strains of xylanase was carried out on Potato Dextrose Agar (PDA) medium fortified with 1% (w/v) of xylan incubated at 20^{o}C for seven days. Selection for best producer of xylanase was done on the basis of breadth of clear zones observed after flooding the plates with Gram's iodine indicating the hydrolysis of xylan by xylanase around the colonies. The fungus *T. angustata* was found to produce the highest amount of xylanase followed by *Pseu. roseus and P. canesence* in that order. The cold-active xylanase-secreting ability of the fungal species was verified by incubating them in xylanase producing medium at 20^{o}C. Consequently, *T. angustata*, *Pseu. roseus* and *P. canesence* were found to produce xylanase activity equal to 11.0 IU/ml, 7.0 IU/ml and 5.9 IU/ml respectively. This is the first report of the fungus *T. angustata* having cold-active xylanases producing ability.

Key words: *Cold active xylanase, Xylan, Penicillium canesence, Truncatella angustata, Pseudogymnoascus roseus, Screening*

I. INTRODUCTION

Xylanases (O-glycoside hydrolases, EC 3.2.1.8) catalyze the hydrolysis of xylan, the major hemicellulose component in plant cell walls. Depending on its origin, the structure of xylan can differ to a great extent [1]. Xylan is a branched heteropolysaccharide constituting a backbone of 1,4 linked xylopyranosyl units substituted with arabinosyl, glucuronyl and acetyl residues [2]. The hydrolysis of the xylan backbone is basically carried out by endoxylanases (EC 3.2.1.8) and β-xylosidases (EC 3.2.1.37) along with a diversity of debranching enzymes including, α-L-arabinofuranosidases, α-D-glucuronidases and acetyl esterases [3].

Xylanases are a group of microbial enzymes that have attained great interest in recent times due to their biotechnological potential in a number of industrial processes, for example, in the production of xylitol and ethanol [4], in food [5], cellulose and paper industries [6], in the manufacturing of oligosaccharides [7], liquid fuels, cellular proteins and other chemical substances [8], and in poultry, pork and caprine feeding [9]. Cold-active enzymes on the other hand are also gaining importance as they have potential to save energy since they are produced and applied at low temperature. These enzymes have reduced cost of production as there is least care for controlling contamination at lower temperature [10].

Potentially, filamentous fungi are best producer of xylanases from the industrial point of view as they are efficient secretor, capable of producing high levels of extra cellular enzymes and cultivated easily [11]. Therefore, screening of naturally occurring fungal species is an excellent way to obtain novel and better source of enzymes in question for commercial applications [12]. The present study was undertaken to find novel fungal species as source of cold-active xylanases.

II. MATERIALS AND METHODS

Chemicals

All the chemicals used were of analytical grade. Potato Dextrose Agar was obtained from Hi-media, Mumbai, India. Birch wood xylan was purchased from Sigma chemicals Co.,USA.

Microorganism

Psychrotrophic fungal isolates, *Penicillium canesence*, BPF4 [13], *Truncatella angustata* BPF5 [14] *Pseudogymnoascus roseus*, BPF6 [13] were obtained from laboratory stocks.

The fungal strains were maintained on Potato Dextrose Agar (PDA) plates at 4°C. The culture was observed daily and fungal growth was sub cultured onto fresh plates of PDA until pure isolates were obtained.

First round of screening for xylanases producing fungal isolates

First round of screening of fungal isolates was done on the basis of their abilities to grow and utilize xylan as sole carbon source. This was carried out by using Czapek-Dox medium containing (g/l): sucrose – 30, $NaNO_3$ – 2.0, K_2HPO_4 – 1.0, $MgSO_4$ – 0.05, KCl – 0.5, $FeSO_4$ – 0.01, xylan – 1.0, Agar Agar – 20 with pH adjusted to 5. After autoclaving at 121°C and 15 lbs pressure, the medium was poured into petri-plates and allowed to solidify. Cavities of 6 mm size were made in the solidified medium and inoculated with 0.1 mm size of fungal colony from 7 day old culture. The plate was incubated at 20 ± 2°C for three days to allow fungal growth [15].

Second round of screening

To confirm the xylanase producing ability, the fungi inoculated on the xylan-medium were allowed to continue growing till ten days at 20 ± 2°C. Afterward, the culture plate was flooded with Gram's iodine. The plate was then washed with sterile distilled water to see the clear zone around colony.

Confirmation test for cold-active xylanase production

Enzyme production was carried out in 50 mL of basal medium, containing Czapek-Dox medium with 1% xylan substrate as carbon source in Erlenmeyer flasks (250 mL). The medium was inoculated with pieces (1 mm^2) of 7 days old colony, followed by incubation at 20°C for 5 days in an orbital incubator shaker with constant shaking (120 rpm).

Extraction of enzymes

The broth was directly filtered through a four layered cheese cloth and the filtrate obtained was centrifuged at 5000 rpm for 10 min at 4°C. The clear supernatant was used as crude enzyme and stored at -20^{o}C until used.

Enzyme assays and protein determination

Xylanase activity was determined as described by [16]. The assay mixture, in a total volume of 2 mL, contains 0.5 mL of 1 mM of xylan in 50 mM citrate buffer (pH 4.8) and 0.5 mL of diluted crude enzyme. The mixture was incubated at 20°C for 30 min. After completion of incubation period, DNS mixture was added, boiled for 5 min and transferred immediately to a cold water bath. Then 20 mL of distilled water was added to the tubes, mixed and the developed colour was measured at 540 nm to estimate the amount of reducing sugars released [17]. The enzymatic activity of xylanase was defined in international units (IU). One unit of enzymatic activity was defined as the amount of enzyme that released 1µmol reducing sugars (xylose) per mL per min.

Protein assay

In addition, the protein assay by Lowry method (1951) was carried out in order to calculate the specific enzymatic activity.

Reproducibility of results

All results are the means of at least three (n=3) independent experiments.

III. RESULTS AND DISCUSSION:

First round of screening for xylanase production

In the present work screening and identification of xylanase producing fungi from laboratory stock was studied. A total of three psychrotrophic isolates *Penicillium canesence* (BPF4), *Truncatella angustata* (BPF5), and *Pseudogymnoascus roseus* (BPF6) were selected to ensure the best producer of xylanase on Czapek-Dox medium supplemented with 1% (w/v) of xylan as sole carbon source and kept at 20^{o}C for three days. All the three fungal species showed positive growth, though rate of growth was different. *T. angustata* showed best growth performance. Most fungal species are known for the secretion of xylanase but species belonging to the genera *Aspergillus* and *Trichoderma* have been reported to produce the enzyme on an industrial scale [18].

Second round of screening for cold-active xylanase production

After a time period of seven days it was found that *T. angustata* (BPF5) demonstrated more vigorous growth on xylanase-medium. It formed broader zone due to hydrolysis of xylan in comparison with those formed by *Pseu. roseus* and *P. canesence* and thus was selected as the potential producer of xylanase (Fig.1). *T. angustata* shows potential xylanase activity and resulting zone of clearance in xylan plate assay as it produced large amount of xylanase. The size of the zone was taken as the measure of the amount of xylanase production. Earlier, xylanase producing fungi were screened from mangrove forest soil on solid medium and eight isolates *Aspergillus sp., Aureobasidium sp., Colletotrichum sp., Fusarium sp., Paecilomyces sp., Guignardia sp., Penicillium sp.,* and *Phomopsis sp.* were selected as positive for xylanase production [19]. Another prospective xylanase producing fungus *Aspergillus candidus* was also isolated from soil sample [20]. Screening of xylanase has been reported from many microbial strains including cold active microbes [21, 22, 23, 24, 25], but no report claiming screening and production of cold active xylanase from *T. angustata* has been reported.

Cold-active xylanase production under SMF

All the three fungi were grown under submerged condition in the xylanase production medium for five days of incubation. The enzyme activity was assayed at 20^{o}C after five days of incubation. The enzyme activity for *T.*

angustata, *P. roseus* and *P. canesense* was found to be 11.0 IU/mL, 7.0 IU/mL and 5.9 IU/mL (µmol/min/mL) (Fig. 2). The activity is far better than reported earlier, for example the specific activity of xylanase from *Glaciecola mesophila* [22], *Flavobacterium sp.* [21], *Bacillus sp.*HJ2 [24], *Sorangium cellulosum* [26] and *Zunongwangia profunda* [25] has been reported to be 98.31, 142.00, 16.10, 8.91 and 2.98 µmol/min/mg respectively.

Psychrotrophic fungi are thought to have potential to improve the climatic conditions not only by bringing down the increasing temperatures but as well by producing these enzymes themselves at relatively lower temperatures [10].

Fig.1. Xyalanase screening: F4-*P. canesence* (BPF4), F5-*T. angustata* (BPF5), and F6-*Pseu. roseus* (BPF6)

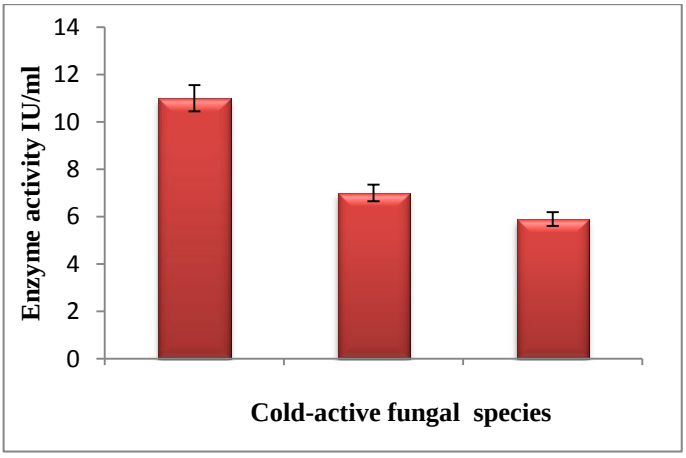

Fig. 2. Xylanase assay after 5 days of incubation at 20°C under SMF

Xylan is the major component of hemicellulose which is the second most abundant polysaccharide. Degradation of xylan into sugars is of much importance for many industries and so is the xylanase enzyme required for its degradation. Fungi are well known agents of decomposition of particularly xylan and cellulose containing organic matter. Xylanases are important industrial enzymes which depolymerizes xylan molecules into xylose units [27]. They have wide applications with potentials for use in the food processing, beverage, livestock feed, paper and pulp, detergent and textile industries as well as in the conversion of agricultural (lignocellulosic) biomass into products with commercial value [28, 29, 30]. With the increasing demand for alternative liquid fuels worldwide, the enzyme is used for enzymatic hydrolysis of lignocellulosic biomass in bioethanol production process [31].

IV. CONCLUSION

In conclusion, the results obtained indicate that the selected fungal species yield variable xylanase activities at low temperature. One of the isolate *T. angustata* (BPF5) showed good xylanase activity in comparison to *Pseu. roseus* (BPF6) and *P. canesence* (BPF4) as determined by the clear zone of hydrolysis around xylose-supplemented medium. The cultivation systems can easily be modified to enhance the productivity of the enzyme formation by the fungus, which will facilitate the scale up processes for mass production.

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