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Screening and evaluation of soil fungal isolates for xylanase production

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Abstract
Fungi are well known for their ability to excrete enzyme into the environment for their growth and survival. 32 fungal strains isolated from soil of social forest area of Bhilai Township were screened for xylanase production. Out of the 17 primary positive xylanase strains showing clear zones around growing colony, 03 good producers were identified as *Aspergillus niger* (AS012), *Gliocledium* sp (GS005) and *Trichoderma viride* (TS007). Solid state fermentation conditions were found to support optimally over Liquid state condition and *Aspergillus niger* (AS012) was appeared as best producer among them. Further studies were carried out on *Aspergillus niger* to standardize best natural substrate for xylanase production under solid state fermentation condition. Wheat bran supported the best followed by whole oat powder and paddy straw respectively.

Keywords: Soil fungal isolates, xylanase, Screening

INTRODUCTION

Xylan is the major constituent of hemicellulose and is the second most abundant renewable resource with a high potential for degradation into useful end products (Goheen, 1982). Microbial xylanases (1,4-β−D-xylan xylanohydrolase, EC 3.2.1.8) are the preferred catalysts for xylan hydrolysis due to their high specificity, mild reaction conditions, negligible substrate loss, and side product generation. However, the cost of the enzymatic hydrolysis of a biomass is one of the main factors limiting the economic feasibility of this process. Therefore, the production of xylanases must be improved by finding more potent microbial strains or by inducing mutant strains that can excrete greater amounts of enzymes, or both (Dekker and Richards, 1976). Filamentous fungi are particularly interesting producers of xylanases since they excrete the enzymes into the medium and their enzyme levels are much higher than those of yeast and bacteria (Steiner *et al*., 1987).

Although bacterial xylanase were reported to have higher values which are beneficial from the view point of applying in biobleaching process but xylanase produced from fungi usually has higher activity (Kulkarni et al., 1999 and Subramaniyan and Prema, 2002). Higher activity of crude enzyme is very important especially when the enzyme is to be applied in its crude form not in pure condition. Fungal strains usually prefer solid state fermentation media which has low water content. These media types are one of the most responsible factors for the higher value of crude enzyme activity obtained from fungal strains.

Main objectives of the study included isolation of potential xylanase-producing fungi from soil and to screen strains that secrete the maximum amount of xylanase during growth.

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MATERIALS AND METHODS

Sample – 10g subsoil was collected randomly from different social forest areas of Bhilai Township in sterilized polythene bags and processed within 4-5 days for isolation of fungi.

Isolation and Identification – Fungi were preliminarily isolated by dilution plate method on Czapek Dox Agar medium. Pure cultures of individual isolates were identified by the available literature (Barnett *et al*. 1972; Gilman, 1959 and Ellis, 1971) and further subjected to screening for xylanase production.

Primary screening for xylanase production on Czapek's agar medium – 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): Beech wood xylan (Hi media), 5.0; 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 and after 30 min, washed with 1 M NaCl and were observed for zone of clearance around the fungal growth. Fungal strains, which produced distinct clearing zones around their colonies, were selected.

Secondary screening for xylanase production on Czapek's agar medium – The cultivation was carried out in a Czapek Dox liquid medium containing (1%) xylan as the sole carbon source. An Erlenmayer flask (250 ml) containing 100 ml of the growth medium was cultured for 5 days at 28 °C on an orbital shaker set at 250 rev min-1. The mycelia were removed by filtration and the filtrate obtained was used as enzyme after partial purification.

Partial purification of xylanase –Previously chilled Ethanol was added drop wise to the culture filtrate (100 ml) at 4 °C with continuous stirring to a final concentration of 75%, and then the solution was left at –20 °C for 24 h. The resulting precipitate was collected by decantation and centrifugation. The precipitate was then dissolved in 30 ml of a phosphate buffer (50 mM, pH 5.0) and the

concentrated solution dialyzed against the same buffer (pH 5.0) overnight at 4 °C (Widjaja1 *et al*., 2009).

Enzyme assays – Xylanase activity was determined by mixing 0.9 ml of 1% (w/v) beech wood xylan (prepared in 50 mM Na-citrate buffer, pH 5.3) with 0.5 ml of suitably diluted enzyme and 0.5 ml of substrate was incubated at 37°C for 30 min. (Bailey *et al.,* 1992). The reaction was stopped by addition of 1.5 ml of 3, 5-dinitrosalicylic acid (DNS) and the contents were boiled for 5 min (Miller, 1959). After cooling, the colour developed was read at 540 nm. The amount of reducing sugar liberated was quantified using xylose as standard. One unit of xylanase is defined as the amount of enzyme that liberates 1 mmol of xylose equivalents per minute under the assay conditions (Nair *et al*. 1983).

Optimization of fermentation condition – The isolates showing positive response in secondary screening were used for optimization of fermentationcondition. The composition of the mineral salts solution was (g.L-1): KCl, 0.5; MgSO4.7H2O, 0.5; (NH4)2HPO4, 2.5; NaH2PO4, 0.5; CaCl2.2H2O, 0.01; FeSO4.7H2O, 0.01; ZnSO4.7H2O, 0.002 and beech wood xylan, 1.0. The pH was adjusted to 5. The medium was then autoclaved for 20min at 121ºC (15 lbs). After cooling, the flasks were inoculated with 1 ml of spore suspension containing 100-120 spores/ml, obtained from 7 day-old pure cultures. However, in SSF condition, wheat bran was added. The pH of the medium was adjusted to 5.0. The flasks were incubated at 30ºC on a rotary shaker (100 rpm) for 7 days. After incubation, the medium was filtered through Whatman No.1 filter paper and the filtrate was centrifuged at 10,000 x g rpm for 15 min at 4ºC. The clear supernatant was used as source of xylanase after partial purification.

Standardization of substrate –The fungi were cultured in Erlenmeyer flasks (250 ml) containing 10 g of wheat bran/ rice bran/ corn cobs/ whole oat/ paddy straw/ saw dust (particle size 30 - 50 mm) moistened with 10 ml of mineral salts solution. The medium was then autoclaved for 20min at 121ºC (15 lbs). After cooling, the flasks were inoculated with 1 ml of spore suspension containing 100-120 spores/ml, obtained from 7 day-old pure cultures. After mixing, flasks were incubated at 28ºC under static conditions for 7 days. After incubation, the enzyme was harvested in sodium citrate buffer (50 mM, pH 5.3). The fermented slurry was filtered through cheese cloth and centrifuged at 10 000 x g for 20 min at 4°C. The clear supernatant was used for enzyme assay after partial purification.

RESULT AND DISCUSSION

Altogether 32 fungal species were isolated from three different sites of the study area (Table-1). However site wise variation was not found significant, but the isolates were maintained separately and given individual codes. Screening for xylanase activity on agar medium revealed that 17 strains were positive for extracellular xylanase production (Table-1). The positive isolates were subjected to secondary screening using partially purified extracellular enzyme to degrade the pure substrate for xylose production. Out of the total 17 isolates, 11 were found positive for xylanase production with varying degree of intensities (Table-2). *Aspergillus niger*, *Gliocladium sp.* and *Trichoderma viride* were identified as good producers of enzyme.

Further studies were carried out with these three isolates for optimization of fermentation conditions. It was observed that all the isolates responded well under solid state fermentation condition over liquid state one (Table-3). Allah Antoine Assamoi *et al.* (2008), Nair *et al.,* (2008), Xian-Jun Dai *et al*. (2011) and Nan Jin *et al.,* (2012) also supported the use of solid-state fermentation bioreactor and suggested that it is not only good for control of microbial fermentation that affects the growth of some important parameters, but also for substantially increase production.

Aspergillus niger (AS012) appeared as best producer with high activity among all. Kavya and Padmavathi (2009) also proved through their studies that *A. niger* is highly potential and useful for xylanase production.

Studies for standardization of natural substrate for xylanase production revealed that wheat bran is best supporter among all with highest production of enzyme units (23.52) followed by whole oats (19.21) and paddy straw (16.24) respectively. Okafor et al., (2007) also reported that wheat bran holds greatest promise for low cost production of the xylanase enzyme. However, Svarachorn (1999) resulted rice straw as best supporter under solid state fermentation.

Table 1. Diversity of soil fungi from study site and primary screening for Xylanase activity

S.No.	Name of Fungal Species	Site A	Site B	Site C	Xylanase Activity
1.	*Alternaria alternata*	+	+	-	-
2.	*A. tritici*	+	-	-	+
3.	*A. soleni*	+	+	-	-
4.	*A. brassicola*	-	+	+	-
5.	*Aspergillus fumigates*	-	+	+	-
6.	*A. flavus*	+	+	+	+
7.	*A. niger*	+	+	+	+
8.	*A. sulphureus*	+	+	-	+
9.	*A. terreus*	-	+	+	-
10.	*Bipolaris indica*	+	+	+	-
11.	*Botrytis cinereae*	+	+	+	+
12.	*Candida albicans*	+	-	-	-
13.	*Cladosporium clavatum*	+	+	+	+
14.	*C. herbarum*	-	-	+	-
15.	*Cunninghemela sp.*	+	+	+	+
16.	*Curvularia catenulate*	+	+	+	+
17.	*C. geniculata*	+	+	-	+
18.	*C. lunata*	+	-	+	+
19.	*C. tetramera*	-	-	+	-

20.	*Fusarium oxysporum*	-	+	+	-
21.	*Geotricum candidum*	-	+	-	+
22.	*Gliocladium sp.*	+	-	+	+
23.	*Mucor sp.*	+	+	+	+
24.	*Nigraspora sp.*	-	+	+	-
25.	*Penicillium citrinum*	+	+	+	-
26.	*P. fuscum*	-	-	+	-
27.	*Penicillium sp.*	+	+	+	-
28.	*Rhizopus nigricans*	+	+	-	+
29.	*Torula sp.*	+	-	-	+
30	*Trichoderma lignorum*	+	-	-	+
31.	*T. viride*	+	-	-	+
32	*Verticillium sp.*	-	-	+	-
	Total	23	20	22	17

Table 2. Secondary screening for xylanase production

S.No.	Name of the isolate	Xylanase activity
1.	*Alternaria tritici*	-
2.	*Aspergillus flavus*	-
3.	*A. niger*	+++
4.	*A. sulphureus*	+
5.	*Botrytis cinereae*	-
6.	*Cladosporium clavatum*	+
7.	*Cunninghemela sp.*	+
8.	*Curvularia catenulate*	+
9.	*C. geniculata*	+
10.	*C. lunata*	-
11.	*Geotricum candidum*	+
12.	*Gliocladium sp.*	++
13.	*Mucor sp.*	+
14.	*Rhizopus nigricans*	+
15.	*Torula sp.*	-
16.	*Trichoderma lignorum*	-
17.	*T. viride*	++
Total		11

Table 3.Opimization of fermentation condition for three good producers

S.No.	Name & Code of Isolates	Xylanase Production	
		LSF	SSF
1.	*Aspergillus niger* (AS012)	+	+++
2.	*Gliocladium sp* .(GS005)	-	+
3.	*Trichoderma viride* (TS007)	-	+

Table 4. Standerdization of substrate for xylanase production by *Aspergillus niger* (AS012) under SSF condition

S.No.	Substrate	Enzyme units/g
1.	Wheat bran	23.52
2.	Rice bran	12.67
3.	Corn cob	10.41
4.	Whole oat	19.21
5.	Paddy straw	16.24
6.	Saw dust	08.74

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