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Xylanase production from *Aspergillus wentii* using wheat bran as a carbon source

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The production of xylanase by *Aspergillus wentii* in submerged fermentation at different ranges of pH, temperature and time periods was studied. The maximum xylanase production, was obtained at pH 6.0, temperature 40^0C but it was at par with 35^0C and time period of 120hrs. Optimization studies were carried out to find the effect of carbon and nitrogen sources on xylanase production and it was observed that wheat bran xylan medium supplemented with methyl - β - D xylopyranoside and $NaNO_3$ supported good yield of xylanase.

Key words : Xylanase, Liquid state fermentation (LSF), *Aspergillus wentii*, Wheat bran, Static conditions

INTRODUCTION

Agricultural residues contain 20-30% hemicellulosic material, which can be utilized by microorganisms. The main carbohydrate constituent of the lignocellulosic material are cellulose, mannan and xylan. Xylan constitutes the major noncellulosic polysaccharides of primary cell wall of grasses and secondary wall of all angiosperms (Asbah *et al.,* 2000; Diaz *et al.,* 2004).

Xylan can be hydrolyzed to xylose by xylanase (1,4-b-D-glucanxylanohydrolase, EC.3.2.1.8) and b-xylosidase (1,4-b-D-xylan xylohydrolase, EC.3.2.1.3.7). Xylanases have attracted considerable research interest because of their potential industrial applications, including hydrolysis of lignocelluloses to biofuel fermentable sugars, bread making, clarification of beer and juices. The most important application of xylanases is the pretreatment of pulp prior to bleaching in pulp and paper industry (Sandrim *et al*., 2005). A variety of microorganisms including bacteria, yeasts and filamentous fungi have been reported to produce xylanolytic enzymes (Coughlan and Hazelwood, 1993).

Fungal xylanases can be produced using two main methods, solid-state cultivation system and liquid state cultivation systems (Bakir *et al.,* 2001). Using abundantly available agro residues in fermentation processes serve the dual purpose of cost – effective enzyme production and environment security. Wheat bran is a low cost raw material leads to reduction in the culture medium cost that generally range from 4-25% of the total production. In this paper the cultivation conditions for the production of xylanase using *Aspergillus wentii* under liquid state fermentation are reported.

MATERIALS AND METHODS

Chemicals :

Wheat bran was purchased from the local market. Oat spelt xylan (substrate) was purchased from Sigma Chemical Company, USA and other chemicals used were of analytical grade.

Organism and inoculum:*illus wentii* was isolated from coir pith soil samples collected in the Coimbatore region of the state of Tamil Nadu. The fungus was maintained at 4^0C on Potato dextrose agar slants (PDA).The fungus was identified with the help of standard manual (Gilman, 1957). Spore suspensions were prepared by adding 10mL of sterilized distilled water to the test tubes whose surface were gently scrapped with a glass rod. Then 10^6/ml of the spores were inoculated into the medium.

Liquid state fermentation (LSF) :

The production medium (Czapek Dox medium) had the following composition (g/L):Wheat bran-30; $NaNO_2$-20; KCl-0.5; $MgSO_4$,$7H_2O$-0.5;KH_2PO_4-1.0; $FeSO_4$. $7H_2O$-0.01. The conical flasks (250ml) containing 100ml of liquid medium were used for liquid state fermentation. The flasks were inoculated with the spores and incubated at 30^0C for 144 h at static conditions. At the end of fermentation the culture flasks were filtered through Whattman No 41 filter paper and the filterate was centrifuged at 3000 rpm for 30 min and assayed for enzyme activity.

Xylanase assay :

Xylanase activity was determined by the method of

Bailey *et al*. (1992). Oat spelt xylan, 1per cent in 0.05M acetate buffer pH 5.0 was used as the substrate. Xylan solution, 0.5 ml with an equal volume of diluted enzyme solution was incubated at 50^{0}C. After 30 min, the reaction mixture was stopped by adding 0.5ml of 10%TCA.The mixture was centrifuged at 3000 rpm and 1ml of supernatant and 3ml of DNS were added. Incubated for 30 min and read the colour developed at 530nm against the blank prepared in the same manner. The reducing sugar was determined by Dinitrosalicyclic acid method of Miller, (1959). Xylose graph served as the standard. One International unit of enzyme is defined as the amount of enzyme that releases 1µmol of D-xylose per min per ml under the above assay conditions.

RESULTS AND DISCUSSION

Various agricultural residues have been screened for xylanase production. Wheat bran, wheat straw, rice bran, paddy husk, corn cobs, sugarcane bagasse, tamarind seed were widely used by many workers. In the present study nine different agro residues have been tried for xylanase production and amongst them wheat bran was found to produce more xylanase production in *Aspergillus wentii* (Table 1). While other substrates supported very small amount of xylanase production. Similar results were already reported in *Schizophyllum radiatum* (Cavazzoni *et al*., 1989) and in *Aureobasidium pullulans* (Karni *et al*., 1998).

Table 1 : Effect of xylan sources on xylanase production by *Aspergillus wentii*

Xylan sources	Xylanase production (IU/ml)
Rice bran	0.40^c
Wheat bran	0.55^a
Cotton shell	0.33^d
Corn cob	0.22^f
Wheat straw	0.48^b
Rice straw	0.26^e
Tamarind seed	0.26^e
Groundnut shell	0.22^f
Cyanodon dactylon	0.12^g
Mean	0.32

S.E. ± - 0.01; C.D. (P=0.05) – 0.03; C.D. (P=0.01) – 0.04
In a column, means followed by a common letter are not significantly different at the 5% level by DMRT.

In general the xylanase of fungal origin have an acidic pH for production (Wong *et al*., 1988). The majority of xylanase production exhibits near to neutral pH optima for growth and enzyme production (Ball and Mccarthy, 1989). The response of various pH in the culture medium were tested (5.0-8.0). The maximum xylanase production was noticed at pH 6.0 (Table 2).

Table 2 : Effect of pH on xylanase production by *Aspergillus wentii*

pH	Xylanase production (IU/ml)
4	2.50^d
4.5	2.30^{de}
5.0	2.55^d
5.5	4.20^c
6.0	6.50^a
6.5	5.95^b
7.0	4.20^c
7.5	2.12^e
8.0	2.00^e
Mean	3.59

S.E. ± 0.16; C.D. (P = 0.05) – 0.32; C.D. (P = 0.01) – 0.43
In a column, means followed by a common letter are not significantly different at the 5% level by DMRT.

Temperature plays an important role in xylanase production. The optimum temperature for xylanase production by bacteria and fungi varies between 40^{0}C and 60^{0}C (Kulkarni *et al*., 1999) and at 37^{0}C in *Neocallimastic frontalis* (Douglas *et al*., 1989). The effect of temperature for xylanase production was investigated (20-45^{0}C). The present results of temperature indicated that maximum production of xylanase noticed at 40^{0}C but it was at par with 35^{0}C. Increase in the incubation temperature decreased the xylanase production and growth of *Aspergillus wentii* (Table 3). There was no growth and biomass content at the temperature higher than 40^{0}C.

Table 3 : Effect of temperature on xylanase production by *Aspergillus wentii*

Temperature (C^2)	Xylanase production (IU/ml)
30	4.35^c
35	4.95^b
40	4.99^b
50	-*
Mean	4.76

S.E. ± - 0.19; C.D. (P = 005) – 0.40; C.D. (P = 0.01) – 0.55
In a column, means followed by a common letter are not significantly different at the 5% level by DMRT.

Time course of production of xylanase varies from organism to organisms. Maximum xylanase production has been reported from 24-192 hours in various organisms. In *Fusarium verticillioides* (Saha, 2001) the enzyme

production reached maximum from 24-48 hours while 144 hours in *Aspergillus ochraceus* (Das and Nanda, 1995). In the present study a sharp increase in xylanase production in *Aspergillus wentii* was noticed upto120 hours. After 120 hours there was a sharp decline (Table 4).

Table 4 : Effect of time period on xylanase production by *Aspergillus wentii*

Incubation (hrs)	Xylanase production (IU/ml)
48	0.50^{c}
72	0.58^{c}
96	0.76^{b}
120	0.95^{a}
144	0.88^{ab}
168	0.75^{b}
192	0.73^{b}
Mean	0.78

S.E. ± - 0.07; C.D. (P=0.05) – 0.14; C.D. (P=0.01) – 0.18
In a column, means followed by a common letter are not significantly different at the 5% level by DMRT.

Despite the increase in knowledge of microbial xylanolytic systems in the past few years, studies on induction and secretion of xylanases are necessary to develop efficient xylanase producers for possible commercial applications. Xylose induction, xylose repression and glucose repression in many fungi and bacteria was reported by many workers. Xylanase induction by other compounds was also studied by many workers.

In the present study, it can be seen that (Table 5) wheat bran xylan medium supplemented with methyl-β-D-xylopyranoside alone supported good yield of xylanase. Cellulose and glucose gave the lowest yield of xylanase. Xylose supported more enzyme production than other sugars tested. Induction of xylanase by xylose was noticed in the present study, which is in accordance with the results reported in *Aureobasidum pullulans* (Karni *et al.,* 1993). Low levels of xylanase synthesis in the absence of xylan and xylose in the growth medium has been observed for most of the xylanolytic organisms because a basal amount of xylanases needed to hydrolyse xylan in the medium to from monomer and short oligomers which only can enter into the cell (Anthony *et al.*,2003).

Organic and inorganic nitrogen sources play an important role in the production of xylanase. In the present study various organic and inorganic nitrogen sources were tested for xylanase production (Table 6). The $NaNO_3$ was able to promote optimal xylanase production. These results are similar to the findings of Maheswari and Kamalam (1985) in *Melanocarpus albomyces*.

Table 5 : Effect of carbon sources on xylanase production by *Aspergillus wentii*

Carbon sources	Xylanase production (IU/ml)
Glucose	0.06^{g}
Fructose	0.31^{f}
Xylose	0.80^{b}
Sucrose	0.74^{bc}
Maltose	0.40e
Xylan(oat spelt)	0.51^{d}
Wheat bran xylan	0.70^{c}
Methyl-β-D-xylopyranoside+wheat bran xylan	1.00^{a}
Methyl-β-D-xylopyranoside	0.81^{b}
Cellulose	0.10^{g}
Mean	0.54

S.E. ± 0.03; C.D. (P=0.05) – 0.07; C.D. (P=0.01) – 0.09
In a column, means followed by a common letter are not significantly different at the 5% level by DMRT.

Table 6 : Effect of nitrogen sources on xylanase production by *Aspergillus wentii*

Nitrogen sources	Xylanase production (IU/ml)
$NaNO_3$	2.26^{a}
KNO_3	1.95^{b}
KNO_2	1.75^{e}
NH_4NO_3	1.46^{d}
$NaNO_2$	1.20^{e}
Peptone	1.01^{f}
Glutamine	1.70^{c}
Mean	1.62

S.E. ± 0.07; C.D. (P=0.05) – 0.13; C.D. (P=0.01) – 0.18
In a column, means followed by a common letter are not significantly different at the 5% level by DMRT

In the present study optimization of xylanase production by *Aspergillus wentii* using agro industrial wastes was considered. Wheat bran, which is inexpensive and abundant, supported the highest activity in liquid state fermentation. Since pure xylan is presently too expensive to be used as industrial substrate for the production of xylanase, the development of wheat bran as a cheap alternative is an attractive substrate.

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