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Effect of Carbon and Nitrogen Sources on Xylanase Production by Mutant Strain of *Aspergillus niger* GCBMX-45

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Abstract: The present study is concerned with the effect of carbon and nitrogen sources and their concentrations on the production of xylanase by mutant strain of *Aspergillus niger* GCBMX-45. High xylanase activities (2350 U/g) were observed when starch (2%) was used as carbon source. $(NH_4)_2SO_4$ (0.2%) was found to be best nitrogen source for optimum enzyme production i.e. 2480 U/g. The production of enzyme reached maximum, 72 hours after inoculation. All the fermentations were carried out at 30°C at 0.4 cm (10g wheat bran) depth of the basal medium.

Key words: Xylanase, *Aspergillus niger*, Xylan degradation, starch, $(NH_4)_2SO_4$

Introduction

Xylanase have been extensively studied and could potentially be employed for the production of hydrolysate from agro-industrial wastes, nutritional improvements of lignocellulosic feeds, processing of food and increasing animal feed digestibility, agro-fiber, biobleaching of kraft paper pulp (Kutsai *et al.*, 2001). Large quantities of agricultural residues accumulate every year which result not only in the deterioration of the environment but also in the loss of potentially valuable material which can be processed such as food, fuel and a variety of chemicals (Someet *et al.*, 2001). Bilgrami and Pander (1992) described that microbial production of xylanase was preferred to plant and animal sources because of easier availability, structural stability and ease of genetic manipulations. Xylanase have been isolated from diversified range of microorganisms including fungi and bacteria (Medeiros *et al.*, 2000). Xylanase used in pulp bleaching must be relatively free of cellulytic enzyme in order to avoid loss of yield and decreased pulp viscosity (Kulkarni *et al.*, 1999; Wu *et al.*, 2000). Microbial xylanses enhanced dough rheological properties as increase in loaf volume that improves its baking performance and so have great importance in cereal industry (Nunez *et al.*, 2001). Enzyme production is also related to the type and concentration of carbon source used (Gawande and Kamat, 2000). Cho *et al.* (1997) studied starch, the best carbon source for maximum xylanase production. Xylanase activity increase in the presence of $(NH_4)_2SO_4$ (Kansoh and Gammal, 2001; Kohli *et al.*, 2001; Chen *et al.*, 2001) In the present study, the effect of different carbon and nitrogen sources at different concentrations were checked for the optimum xylanase production by using mutant strain of *Aspergillus niger* GCBMX-45.

Materials and Methods

Aspergillus niger strain GCBMX-45 was taken from Biotechnology laboratory, Department of Botany, Govt. College, Lahore and was maintained on potato dextrose agar medium (Merck, Germany), pH 4.5, stored at 4°C in a refrigerator. Sub culturing of mould was carried out after 15 days on solidified potato dextrose agar slants (PDA). The conidial inoculum was used in the present study. Conidia from 3-5 days old cultures were used for inoculation. The suspension was prepared by adding 10ml solution of 0.005 % monoxal O.T to the slant having profuse conidial growth on its surface. The inoculating needle was used to break the conidial clumps.

Ten grams of wheat bran was taken in 250ml conical flask and moistened by adding 10ml of distilled water. The flasks were cotton plugged and sterilized in an autoclave at 121°C for 15min and 15lb/inch^{2}pressure. After cooling at room temperature, 1.0 ml conidial suspension was added and incubated at 30°C for 72 hours. Shaken twice daily. After 72h of incubation period 100ml of distilled water was added to each flask. The flasks were then rotated (200 rpm) at the rotary incubator shaker for 1h. After 1h the fermented mash of the flasks was filtered and filtrate was used for the estimation of xylanase. The extract of fermented wheat bran was analyzed for the estimation of xylose (reducing sugar) by using DNS method (Miller, 1959)

Xylanase activity was determined by the method of Somogyi (1952). The colour intensity of dilution was estimated at photoelectric colorimeter using green Wratten filter of 546 nm. One xylanase unit is defined as "the amount of enzyme which release one mole of reducing sugar per minute at pH 7.0 and 30°C". Enzyme activity was expressed as U/g (Wong, 1988).

Results and Discussion

Enzyme production is also related to the type and concentration of carbon source used (Gawande and Kamat, 2000). In the present work wheat bran was used as substrate, which already contained 69% carbohydrates, additional carbon sources were added to increase the enzyme production. So different carbon sources were investigated for xylanase production (Table1). Starch was found to be the best additional carbon source for maximum xylanase production (2300 U/g). This is because xylan is complex medium need more enzyme for its breakage, more powerful growth of mycelium required for its breakage so starch converted to simple sugar as glucose which can easily uptake by fungus for its growth. The minimum enzyme activity was obtained from xylose (325 U/g). Cho (1997) also studied starch, the best carbon source for maximum xylanase production (305.0 u/mL). But it was lesser than the production in present studies

Table1a shows the effect of different starch concentrations (1-5.0%) on xylanase production by mutant strain of *Aspergillus niger* UV-45. There was a gradual increase in xylanase production from 1-2% concentration. Beyond this the xylanase production was decreased. Thus, the optimum concentration of starch for the production of xylanase was 2% because maximum production (2350 U/g) was observed at this concentration. However, when starch concentration was increased up to 5%, the production of xylanase was decreased up to 1000 U/g. This is due to further increase in concentration of carbon source, the activity of enzyme was decreased due to catabolite repression and thus production was decreased. Cho (1997) obtained the best xylanase production

Table 1: Effect of additional carbon sources

Carbon sources(2%)	Xylanase saccharifying activity(U/g)
Xylose	325
Sucrose	1877
Glucose	1895
Starch	2300
Lactose	1400
Maltose	1150
Dextrose	1825

Table 1a: Effect of different of different concentrations of starch

Concentration of starch(%)	Xylanase saccharifying Activity(U/g)
1.0	1150
2.0	2350
3.0	1265
4.0	1035
5.0	1000

Table 2: Effect of different nitrogen sources

Nitrogen sources(0.2 %)	Xylanase saccharifying activity(U/g)
NH_4NO_3	1876
NH_4Cl	1495
$NaNO_3$	1925
$(NH_4)_2SO_4$	2425
Urea	1970

Table 2a: Effect of different concentrations of $(NH_4)_2SO_4$

Concentrations of $(NH_4)_2SO_4$(%)	Xylanase saccharifying activity(U/g)
0.1	1250
0.2	2480
0.3	1305
0.4	1300
0.5	1245

Substrate =Wheat bran (10g).
Moistening agent = Distilled water (pH = 7.0)
Temperature = 30 $\pm$ 1°C.

with 2% starch concentration.

The productivity of xylanase is greatly influenced by both the source and concentration of nitrogen (Kulkarni *et al.*, 1999). In the present work different nitrogen sources NH_4NO_3, NH_4Cl, $(NH_4)_2SO_4$, $NaNO_3$ and urea were evaluated (Table 2). The results showed maximum xylanase production (2425U/g) by using $(NH_4)_2SO_4$ as nitrogen source because it was easy for the mycelium to get nitrogen from it as well as it contains SO_4, which is helpful in the growth of fungus. In the presence of more available nitrogen, the mycelium grows better and its activity also increases. Similar studies were reported by Shamala and Sreekantiah (1986), Gokhale *et al.* (1991), Pinaga *et al.* (1994), Bi *et al.* (1999) Kansoh *et al.* (2001)

Different concentrations of $(NH_4)_2SO_4$ were investigated for xylanase production ranging from 0.1-0.5% (Table 2a). There was increase in the production of xylanase from 0.1 to 0.2%. However, xylanase production declined gradually above this concentration. The maximum xylanase production (2480U/g) was obtained at 0.2% concentration of $(NH_4)_2SO_4$. In lower and higher concentration of $(NH_4)_2SO_4$ the mycelium growth was not well enough to oxidize the medium, therefore, xylanase production decreased. Increased concentration of $(NH_4)_2SO_4$ leads to the toxicity so mold cannot survive therefore, xylanase production decreased. Similar studies were reported by Kansoh and Gammal (2001).

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