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Biosynthesis of Xylanase by UV-Treated Mutant Strain of *Aspergillus niger* GCBMX-45

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Abstract: This investigation describes the biosynthesis of xylanase by UV-treated strain of *Aspergillus niger* GCBMX-45. For this purpose, parental strains of *Aspergillus niger* was UV-irradiated for different time intervals (5-60 minutes). It was found that the strain treated for 45minutes gave the maximum yield of xylanase when different parameters were employed. Among different substrates and carbon sources, wheat bran (10g) and sucrose, respectively gave maximum production. Distilled water as a diluent and incubation period of 72hours at 30^0C were optimized for improved production under solid substrate fermentation conditions.

Key words: Xylanase, UV-treated mutants, *Aspergillus niger*, production, xylan degradation, biosynthesis

Introduction

Increasing competition in the livestock industry has forced producers to cut costs by adapting new technologies and increasing production efficiency (Cheng *et al.*, 1999). Large quantities of agricultural residues accumulate every year which results not only in the deterioration of the environment but also in the loss of potentially valuable material which can be processed to yield a number of value added products such as food, fuel, feed and a variety of chemicals (Someet *et al.*, 2001). Xylanase are the key enzymes for breakdown of xylan since they depolymerize the backbone. They have potential application in biopulping, nutritional improvement of L.C. feedstock production of ethanol, methane, other products and in the processing of food (Wong *et al.*, 1988). Strains of *Aspergillus* are known to produce xylanase on various lignocellulosic substrates (Labeille *et al.*, 1999, Gawande and Kamat, 2000).

The objective of this study is to employ different variables for the biosynthesis of xylanase i.e., selection of the substrate, effect of different diluents and incubation period. For this purpose a UV-irradiated mutant strain of *Aspergillus niger* was used.

Materials and Methods

Organisms used: A UV-irradiated mutant strain of *Aspergillus niger* GCBMX-45 was obtained from

the Mutant Culture Collection of our Labs. The culture was maintained on PDA slants.

Fermentation technique: Ten grams wheat bran was transferred to 250ml conical flask and moistened by adding 10 ml of distilled water. The flasks were plugged with cotton and were sterilized in an autoclave at 121° C for 15 minutes. The flasks were cooled at room temperature and inoculated with 1.0 ml conidial suspension prepared in 0.005% Monoxal O.T. The flasks were then incubated at 30±1°C for 72 hours and shaken twice daily. After 72 hours, 100 ml of distilled water was transferred to each flask. The flasks were then rotated at the rotary incubator shaker (200 rpm) at 30°C for one hour. The fermented broth was filtered and filtrate was used for the estimation of xylanase.

Enzyme assay: The estimation of xylanase was carried out according to the method of Miller (1959). One unit liberates one mole of reducing sugar measured as xylose equivalents from xylan per minute at pH 7.0 and 30°C.

Results and Discussion

Selection of substrate: Selection of substrate is of great importance in solid-state fermentation. The effect of different substrates on the production of xylanase was investigated (Table 1). Maximum production of xylanase (1800 U/g) was obtained by using wheat bran as substrate; where as other substrates such as wheat straw (1250U/g), rice husk (1175U/g), sunflower meal (1300U/g), bagasse (1275 U/g), soybean meal (1175U/g) and news paper (900 U/g) gave low enzyme production. It is due to the fact that wheat bran provides adequate amount of nutrients, proteins 1.32, carbohydrates 69.0, fats 1.9, fibre 2.6, ash 1.8, Ca 0.05, Mg 0.17, P 0.35, K 0.45, S 0.12, various amino acids as well as porosity for oxygen supply. Polygilenia *et al.* (1989) reported that

Table 1: Selection of substrate for the production of Xylanase by mutant strain of *Aspergillus niger* GCBMX-45

Substrates	Xylanase activity (Units/g)
Rice straw	1250
Wheat straw	1250
Rice husk	1175
Wheat bran	1800
Sunflower meal	1300
Bagasse	1275
Soybean meal	1175
News paper	900

Moistening agent = Distilled water pH = 7 Temperature = 30 ± 1°C

Table 2: Effect of various diluents on xylanase production by mutant strains of *Aspergillus niger* GCBMX 45

Diluents	Xylanase activity (Units/g)
0.1N HCl	1400
0.01N HCl	1225
Distilled water	1825
Tap water	1500
Mineral salt solution	1450

Substrate = Wheat bran Temperature = 30±1°C

Table 3: Effect of Incubation time on xylanase production of Xylanase by *Aspergillus niger* GCBMX 45

Incubation period (hour)	Xylanase activity (Units/g)
8	750
16	875
24	1680
32	1150
40	1050
48	1200
56	1250
64	1300
72	1850
80	1450
88	1350
96	1300
104	1250
112	1175
120	1000

Substrate= Wheat bran, Temperature= 30±1 °C

wheat bran contains 8.04% cellulose and rest of lignin and xylan. Large quantities of xylan and increased surface area of wheat bran provides optimum support for xylanase production. Similar kind of work has also been reported by other workers (Haq *et al.*, 1993; Pal *et al.*, 1998).

Effect of different diluents: Wheat bran was moistened with different diluents (Table 2). The enzyme productivity was maximum (1825U/g) with distilled water and minimum (1225U/g) with 0.01 N HCl. Bi *et al.* (1999) obtained maximum xylanase production (92.96U/ml) with distilled water but this study is more significant due to high enzyme units (1825U/g) as compared to them. Reese

et al. (1969) and Haq *et al.* (1993) found mineral salt solution to be better inducer of high production of enzyme. But in our finding, use of distilled water as moistening agent gave better results as compared to mineral salt solution.

Time course study during xylanase biosynthesis: The rate of enzyme synthesis by mutant strain of *Aspergillus niger* UV-45 was investigated The flasks were incubated at 30 °C for 8-120h (Table 3) after spore inoculation. Concentration of reducing sugar showed a significant increase in enzyme production with increase in time, which was presumed to be due to the rapid hydrolysis of xylan in the medium. The maximum production (1850U/g) of enzyme was obtained after 72hrs of incubation. Further, increase in incubation period resulted in the decreased enzyme production. The decrease in enzyme production may be because the susceptible portion of xylan molecules was rapidly digested and only crystalline portion was left behind which cannot be used by the organism for the production of enzyme. This finding is in accordance with the work of Roose (1963) and Jing *et al.* (1998). Gawande and Kamat (2000) studied maximum xylanase activity (26.7IU/ml) after 60 hrs of incubation period by *Aspergillus niger* but the enzyme productivity in their case was less as compared to our findings.

Mutant can raise the status of microorganisms to overproduce the enzymes. In this practical study, the mutant strain of *Aspergillus niger* GCBMX-45 gave encouraging results. Some more study is required to further enhance the xylanase activity under laboratory conditions.

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