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Studies on the Biosynthesis of Enzyme Xylanase by Submerged Fermentation from *Aspergillus niger* GCBMX-45

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Abstract: The optimization of cultural condition was studied for the production of xylanase by mutant strain of *Aspergillus niger* GCBMX-45. Submerged fermentation was carried out in 250 ml Erlenmeyer flasks. Medium containing (%w/v) $NaNO_3$, 0.1; Tween-80, 0.2; NH_4Cl, 0.1; KH_2PO_4, 0.1; $MgSO_4.7H_2O$, 0.03; $CaCl_2.2H_2O$, 0.1; wheat bran, 2 was used for xylanase production. The enzyme production was maximum (195 U ml^{-1}) at pH 4.5. One percent meat extract was added in medium as an additional organic nitrogen source.

Key words: Xylanase, submerged fermentation, *Aspergillus niger*, biosynthesis, mutant strain, filamentous fungi

Introduction

The productivity of xylanase is greatly influenced by different factors. Cai *et al.* (1997) optimized pH 4.2 for xylanase production from *Aspergillus niger*. The optimum pH of xylanase was 4.6 and it was stable at 3-11 in shake flask culture (Chen *et al.*, 1999). Xylanase with initial activity of 42 U ml^{-1} was found to be thermostable within a pH range of 8.5-9.0 (Kohli *et al.*, 2001). *Aspergillus niger* NCIM 1207 produced higher levels of extra cellular beta glucosidase and xylanase in submerged fermentation, when ammonium sulphate, ammonium di hydrogen-orthophosphate and corn steep liquor were used as nitrogen sources (Gokhale *et al.*, 1991). *Aspergillus niger* produced xylanase with undetectable amounts in submerged fermentation when a mixture of sodium nitrate and nitrite was used as nitrogen source (Gouda, 2000).

Materials and Methods

Aspergillus niger GCBMX-45 was taken from Biotechnology Laboratory, Govt. College, Lahore and it was maintained on potato dextrose agar medium (Merck, Germany). Conidia from 3-5 days old cultures were used for inoculation. The suspension was prepared by adding 10 ml of 0.005% monoxal O.T to the slant having profuse conidial growth on its surface. The sterilized inoculating needle was used to break the conidial clumps. Twenty-five ml of fermentation medium containing (%w/v) $NaNO_3$, 0.1; tween-80, 0.2; NH_4Cl, 0.1; KH_2PO_4, 0.1; $MgSO_4.7H_2O$, 0.03; $CaCl_2.2H_2O$, 0.1; wheat bran, 2 was transferred to each 250 ml cotton plugged conical flask. The flasks were sterilized in an autoclave at 121°C for 15 min and 15lb/$inch^2$ pressure. After cooling at room temperature, 1.0 ml of conidial inoculum was transferred to each flask. The flasks were then placed in a rotary incubator shaker (Gallen Kamp PLC, UK) rotating at 160 rpm (30°C) for 48 h.

After 48 h, the fermented broth was filtered and filtrate was analyzed for the estimation of xylanase. Duplicate test tubes were marked A and B. In both of these test tubes, 1.0 ml enzyme filtrate was added, then 0.5 ml, xylan (1.0%) was added along with 0.5 ml of distilled water to each test tube. The test tubes were then placed in an incubator at 30°C for 1 h. After 1 h, 2.0 ml of DNS reagent was added to each test tube and the tubes were heated in boiling water bath for 5 min. The tubes were cooled at room temperature and diluted up to 20.0 ml with distilled water (Miller, 1959). A blank was also prepared in the same manner as mentioned above, but replacing the enzyme extract with 2 ml distilled water. The colour intensity was estimated at 575 nm in a spectrophotometer.

Results and Discussion

The maintenance of a favourable pH is very essential for the successful production of xylanase. Effect of different initial pH (3.5 to 8.0) on the biosynthesis of xylanase by *Aspergillus niger* showed that maximum production of enzyme (110.0 U ml^{-1}) was achieved when the initial pH of basal medium was kept at 4.5 (Fig. 1). When the pH was increased or decreased other than 4.5, the production of xylanase was gradually decreased. It might be due to that alkaline pH has inhibitory effect on the growth of *Aspergillus niger* and subsequently enzyme production. However, pH 4.5 was selected for the production of xylanase by *Aspergillus niger* GCBMX-45. Ilieva *et al.* (1995) found optimum pH for xylanase production in the range of 3.5- 4.0, while Cai *et al.* (1997) optimized the pH 4.2 for xylan ase production and Chen *et al.* (1999) found the optimum pH 4.6 for synthesis of xylanase.

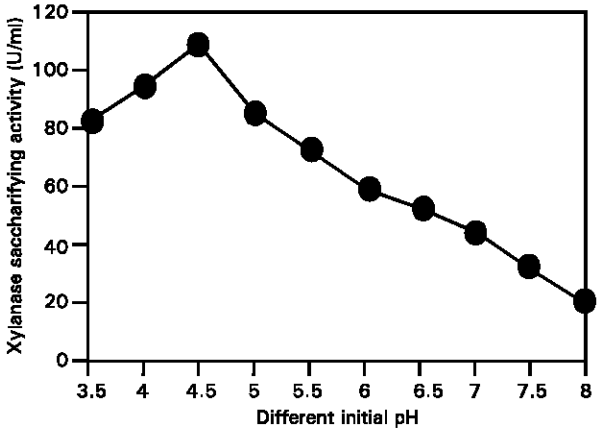

Fig. 1: Effect of different initial pH of basal medium on the production of xylanase by *Aspergillus niger* GCBMX-45.

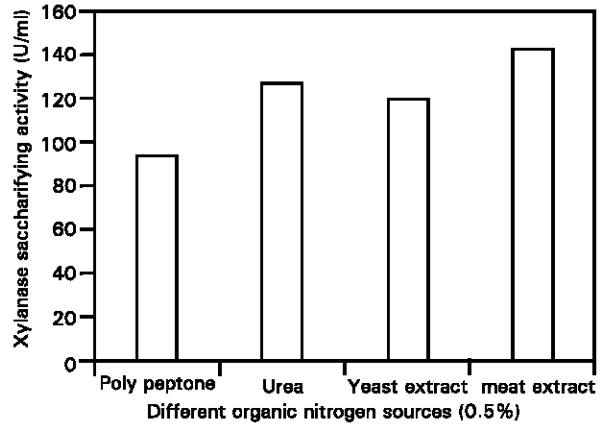

Fig. 2: Effect of different additional organic nitrogen sources on the production of xylanase by *Aspergillus niger* GCBMX-45

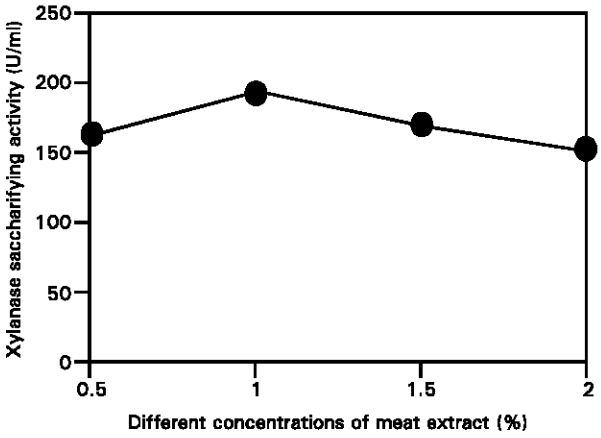

Fig 3: Effect of different concentrations of meat extract on xylanase production by *Aspergillus niger* GCBMX-45.

The production of xylanase is greatly influenced by the addition of different organic nitrogen sources (Kulkarni *et al.*, 1999). In this study, the effect of addition of different nitrogen sources was tested for the production of xylanase. The organic nitrogen sources such as poly peptone, urea, yeast extract and meat extract were added to the fermentation medium at 0.5% level. Among all the nitrogen sources tested, meat extract gave maximum production of xylanase (160.0 U ml^{-1}). However, other nitrogen sources gave insufficient production of xylanase (Fig. 2). Cai *et al.* (2000) have reported that peptone is excellent organic nitrogen source. But in this study, meat extract gave maximum results. It might be due the type of fungal strain and cultural conditions necessary for the production of the xylanase. Thus, meat extract was used as an additional organic nitrogen source for the production of xylanase by *Aspergillus niger* GCBMX-45.

The optimization of concentration of meat extract for the production of xylanase by *Aspergillus niger* GCBMX-45 was also undertaken. The meat extract at a level of 0.5-2.0% was tested for the production of xylanase (Fig. 3). The production of enzyme was found to be maximum (195.0 U ml^{-1}) when 1.0% meat extract was added to the fermentation medium. Further increase in concentration of meat extract, resulted in the decreased production of xylanase. It might be due to that nitrogen sources at higher were toxic for the growth of fungi as well as for the xylanase production. Thus, meat extract at the concentration of 1.0% was optimized for the production of xylanase.

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