

## ABSTRACT

The present study is concerned with the selection of a potent strain of *Aspergillus niger* and optimization of the cultural conditions for the biosynthesis of amyloglucosidase. About 150 strains of *A. niger* were isolated from soils of different habitats. Isolate No. 52 producing enzyme 7.46 U/ml/min was selected and assigned the name BT. The cultural conditions were optimized for the enzyme production. Five culture media were tested for maximum amyloglucosidase production in 250 ml shake flask. The culture medium M2 containing (g/l) Raw starch 10.0, lactose 10.0,  $(\text{NH}_4)_2\text{SO}_4$  5.0,  $\text{MgSO}_4 \cdot \text{H}_2\text{O}$  2.0,  $\text{CaCl}_2 \cdot \text{H}_2\text{O}$  2.0,  $\text{KH}_2\text{PO}_4$  1.50,  $\text{K}_2\text{HPO}_4$  0.1, Distilled water to make final volume 1000ml (pH 5.5) was found to be the best medium for the maximum amyloglucosidase production (11.05 U/ml/min). 50 ml/250ml flask was found to be optimum volume of the medium and the enzyme production was increased to 11.90 U/ml/min. Optimum temperature was 30°C as the production of the enzyme following the growth of the organism was found to be maximum (12.18 U/ml/min). The production of the enzyme was optimum (13.28 U/ml/min) after 72 h of incubation, with the initial pH of the medium 5.0. 2% Starch with 1% glucose as an additional carbon source gave maximum amyloglucosidase production (14.21 U/ml/min). Addition of 0.3% ammonium sulphate in the fermentation medium increased the enzyme production (14.68 U/ml/min). While 2% spore inoculum showed best amyloglucosidase production (14.47 U/ml/min).

The strain was improved by the alternate treatment of the parent strain with ethidium bromide and EMS. The mutant strain M4 120 produced an increased amount of amyloglucosidase (18.84 U/ml/min). The cultural conditions, were also optimized for mutant strain of *Aspergillus niger* M4 120 to obtain maximum enzyme production. The culture medium M2 produced maximum enzyme (19.49 U/ml/min). With 50 ml volume of the fermentation medium, amyloglucosidase production increased (20.32 U/ml/min). The temperature, 30°C was optimum and enzyme production was maximum at this temperature (20.30 U/ml/min). After 72 h of incubation amyloglucosidase reached its maximum level

(20.46 U/ml/min). The initial pH 5.0 was found to be best with the enzyme production (21.86 U/ml/min). Starch was the best carbon source and at 2% starch concentration the productivity of the enzyme increased to 22.84 U/ml/min. When 1% glucose was added as the additional carbon source along with starch still an increased amount of enzyme production was obtained (24.13 U/ml/min). Different nitrogen sources of organic and inorganic nature were tested for the enzyme production. Ammonium sulphate was found to be the best nitrogen source. The enzyme production increased with the addition of ammonium sulphate to 24.16 U/ml/min of amyloglucosidase. When 0.4% concentration of ammonium sulphate was added to the fermentation medium the enzyme production increased to its maximum level (25.29 U/ml/min). Spore inoculum was found better as compared to the vegetative inoculum. With 2% spore inoculum maximum amyloglucosidase production was achieved. Scale-up studies were carried out in a stirred fermentor of 7.5 litres capacity. The production of the amyloglucosidase was maximum when the volume of the medium was 60% (4.5 litres), the speed of agitation was 200 rpm and the aeration rate was maintained at  $1.0 \text{ l l}^{-1} \text{ min}^{-1}$  exhibiting 25.15 U/ml/min of amyloglucosidase. When 4% inoculum was added the maximum enzyme production (25.28 U/ml/min) was achieved after 48 h. The optimum initial pH of the medium was found to be 5.0.

After the optimization of the cultural conditions in the stirred fermentor, partial purification of amyloglucosidase was performed by ammonium sulphate precipitation. The enzyme activity was more in the range of 40-70 % saturation level. The specific activity of amyloglucosidase increased after the partial purification and the maximum specific activity was achieved at 70% ammonium sulphate saturation (21000 U/ml/min). Sodium dodecylsulphate polyacrylamide gel electrophoresis was run to determine the molecular weight of amyloglucosidase. The molecular weight of partially purified amyloglucosidase was found to be 65 KDa approximately.

The characterization of the enzyme was done. The optimum amyloglucosidase activity was obtained at pH 4.75,  $60^{\circ}\text{C}$  after 60 min at 5% starch concentration.