## Abstract

The present study, deals with the isolation, screening and selection of *Aspergillus oryzae* for the alpha amylase production. Seventy eight isolates of *A. oryzae* were isolated from different soil samples. The strains were initially selected qualitatively on starch agar medium and screened quantitatively for enzyme production in shake flasks and a strain producing 130 ±0.1U/ml of enzyme was selected which was assigned the code IIB-30. The selected strain was subjected to physical and chemical mutagenic treatments in order to improve its amylolytic potential. During the treatments, isolates were qualitatively and quantitatively screened. Among these, EMS-18 exhibited the highest enzyme activity (347±1.2 U/ml). This mutant showed 2.6 fold increased activity over the parental strain in terms of enzyme production. The cultural conditions and nutritional requirements of the selected strains (both wild and mutant) were optimized in 250 ml Erlenmeyer flasks prior to scale up studies in a fermenter.

Six different fermentation media were evaluated for the alpha amylase production by both wild and mutant strains of *A. oryzae* in shake flasks fermentation. Of all the media, M4 containing (g/l); starch 20, yeast extract 8.5, $NH_4Cl$ 1.3, $MgSO_4.7H_2O$ 0.12, $CaCl_2$ 0.06 gave maximal enzyme production i.e., 168±2 (wild) and 385±2 (mutant) which was highly significant ($p \leq 0.05$). The effect of incubation temperature, initial pH, volume of media and inoculum size was investigated on the enzyme production. The optimal enzyme production was obtained at 30°C, pH 5, volume, 10 % and inoculum size 4 %, by both wild and mutant strains. The rate of fermentation was also studied and the highest yield of enzyme was obtained 72 h after inoculation.

Corn starch (2 %) and lactose (1.5 %) as carbon sources while, ammonium sulfate (0.3 %) and peptone (0.2 %) as nitrogen sources were also optimized. Different surfactants were added to the fermentation media and Tween 80 at the level of 0.1% was found to be the best for enzyme production.

The scale up studies for alpha amylase production was carried out in a 7.5 L stirred fermenter. The rate of fermentation for enzyme production by both wild and mutant strains was investigated.) It was found that the enzyme production increased gradually and reached maximum (335 U/ml and 608 U/ml) after 64 h (wild) and 48 h (mutant). The kinetic depiction of results showed optimal fermentation period for enzyme production to be 64 h and 48 h, respectively. The other cultural conditions such as initial pH (5), aeration level (1.5 vvm), dissolved oxygen (15 %), inoculum size (10 %) and agitation intensity (200 rpm) were optimized for enzyme production.

The fermented broth was subjected to ammonium sulfate precipitation at different saturation levels (20-90 %). The optimum level of ammonium sulfate saturation was found to be 70 % that gave 1.3 fold purification. By using Sephadex-DEAE column, the active fractions were eluted using 0.05 M Tris-HCl buffer containing 0.30 M NaCl at pH 7.5. The molecular weight of alpha amylase was found to be 48 kDa on SDS-PAGE after gel filtration. A total of 9.5 fold enzyme purification was accomplished. The effect of time, temperature, pH and metal ions on purified enzyme was also investigated and maximum activity was achieved after 30 min at 40ºC and pH 5 in the presence of $Ca^{+2}$ ion.