ABSTRACT

A novel α- amylase gene of *Thermotoga naphthophila* was successfully cloned and expressed into *E. coli*. For this purpose two strains of *E. coli* DH5α and BL21 were used for cloning and expression, respectively. Vector PTZ57R/T was used as cloning vector and pET- 21a (+) was used as expression vector. To obtain optimal expression of α- amylase, conditions for the growth of recombinant *E. coli* were optimized by exploring different parameters e.g. temperature, pH, addition of inducer and time of induction. The optimum pH value for maximal expression was 7.0 and optimal temperature for maximum expression of α- amylase was 37 °C. Heat treatment was used for the partial purification of recombinant enzyme which was further purified by ammonium sulphate precipitation followed by the anion exchange chromatography to achieve maximum purity of recombinant enzyme. SDS-PAGE (sodium dodecyl sulphate polyacrylamide gel electrophoresis) was carried out for the investigation of molecular weight of recombinant enzyme, which was calculated 64.5 kDa after electrophoresis. Considerable stability was shown by the enzyme at pH vale of 7.0 and at temperature of 100 °C. The activity of enzyme was decreased considerably in the presence of EDTA and Cu^{+2} at lower concentration but entire activity of enzyme was lost at higher concentration of both. The presence of calcium ions increased the activity of enzyme up to 145% which indicates that recombinant enzyme was metaloenzyme in nature. The effect of different organic solvents (methanol, ethanol, n-butanol, acetone and isopropanol) was also explored and results showed no considerable effect on activity by these organic solvents. Upon consumption of soluble starch as substrate, under optimized condition enzyme activity was 4.0 U/ml/min. The recombinant α- amylase was functional and produce maltose upon the hydrolysis of starch.