ABSTRACT

In this study, α-amylase gene of Thermoanaerobacterium thermosaccharolyticum ATCC 7956 (DSM 571) was cloned and expressed in E. coli BL21 (DE3), pET-22b(+) was used as cloning and expression vector. An ORF of 1.6 kb α-amylase gene was used for amplification. Amplification was carried out at annealing temperature of 54°C. Following amplification, double restricted amplified gene ligated with double restricted pET-22b(+) was transformed into E. coli BL21 (DE3). After successful transformation, further expression was studied followed by 0.5 mM IPTG induction. The recombinant α-amylase was partially purified by using heat treatment method followed by ammonium sulfate precipitation and ion exchange chromatography. The molecular mass of protein was estimated to be almost 66 kDa by SDS-PAGE, α-amylase was found to be substrate specific. The maximum activity was found at 80°C on pH 8.0. αamylase was found to be stable in pH ranging from 5-9. The results found from current study made novel thermophilic α-amylase of Thermoanaerobacterium thermosaccharolyticum as an important applicant for industrial use.