ABSTRACT

In this study, β-galactosidase gene (BFT35_08785) of *Thermoanaerobacterium thermosaccharolyticum* (DSM 571) was cloned. It was expressed in *Escherichia coli* BL21 (DE3). The expressed enzyme was characterized after purification. The gene was amplified and by using expression vector pET-22b(+), it was cloned in *E. coli* BL21 (DE3). For the screening of positive clones, colony PCR and single and double restriction of recombinant plasmid, by using NcoI and BamHI, was performed. For the expression of gene, it was induced with IPTG and the expression was optimized by optimizing the growth conditions e.g. temperature, pH, IPTG concentration, optical density at the time of induction, and induction time. To purify the enzyme, it was treated with heat followed by immobilized metal ion affinity chromatography with 35.66-fold purification. Its specific activity and recovery was 118.75 U mg^{-1} and 50.44 %, respectively. Its molecular weight estimated by SDS-PAGE (Sodium dodecyl sulfate polyacrylamide gel electrophoresis) was 71,602 Da. The enzyme gave maximum activity at 70 °C pH 6.5. The enzyme was stable over 50 to 90 °C and pH of 5 to 9. The enzyme activity was enhanced by Mn^{2+} indicating that it is a metalloenzyme. EDTA and SDS decreased the activity of enzyme. The addition of 10 to 40 % organic solvents had no prominent effect on activity of enzyme. These properties show the potential of this novel β-galactosidase for use in food and dairy industry.