ABSTRACT

With an increasing demand of an alternative source of energy to run industries and transportations, thermo-stable cellulases are considered to be important enzymes for the saccharification of cellulosic material. β-glucosidase is an essential enzyme which belonged to cellulases and plays a key role in the degradation of cellulosic biomass and in many other biological processes. Consequently, β-glucosidase gene encoding for 629 amino acids from highly thermo-stable *Clostridium clariflavum* DSM 19732 was cloned in pET-21a(+) vector and expressed in *E. coli* BL21 (DE3) CodonPlus. An intracellular β-glucosidase protein displayed a single band of molecular size of 61.1 kDa on SDS-PAGE after single step purification by Ni-TED affinity chromatography. The purified enzyme β-glucosidase showed an optimal residual activity at pH 7 and 80°C temperature. The enzyme was relatively stable over a range of pH (4.5-7.5) and temperature from 60-80°C. Enzyme activity of β-glucosidase was not much affected by Mg^{2+}, Ca^{2+} and K^{2+} while Na^{2+}, Cu^{2+} and Zn^{2+} ions inhibited its enzymatic activity. 10%, 20% and 30% of organic solvents including absolute ethanol, acetone, methanol, n-butanol and iso-propanol drastically inhibited the enzymatic activity of β-glucosidase while maximum residual activity was increased about 1% in the presence of Tween-20, 8% in the presence of 10% β-marcaptoethanol and 4% increase in the presence of 10% Triton X-100. All these significant properties make β-glucosidase gene biotechnologically and industrially important candidate for the useful biological process.