

ABSTRACT

A thermophilic β -glucosidase gene of a eubacterium *Clostridium thermocellum* was expressed in *E.Coli* strain BL-21. The cloned enzyme was purified by heat treatment, ammonium sulfate precipitation, anion exchange chromatography followed by cation exchange chromatography using UNOsphere Q and S column. The specific activity for the purified enzyme was found to be 227964.28 U/mg and its molecular weight was determined as 51.8KDa on SDS-PAGE. The enzyme worked best at pH 6.0 and temperature of 60°C. Mn^{+2} , Ca^{+2} , Co^{+2} , EDTA and Pb^{+2} significantly increased the enzyme activity. Cd^{+2} , Mg^{+2} , Zn^{+2} , Cu^{+2} , Fe^{+2} on the other hand had an inhibitory effect on the activity whereas Hg^{+2} drastically reduce the activity of purified enzyme. The estimated K_m and V_{max} values for p-nitrophenyl β -D-glucopyranoside were 6.94 mM and 26945 μ mol/mim/mg respectively. It showed maximum activity with pNP- β -D-fucopyranoside on incubation with synthetic glucosides. Because of its maximal activity at higher temperature, this enzyme display potential as a catalytic tool for biotechnological application.