Abstract

Anthropogenic activities have led to a drastic shift from natural fuels to alternative renewable energy reserves that demand the use of heat-stable cellulases. Cellulose 1,4-β-cellobiosidase/cellobiohydrolase is an indispensable member of cellulases that play a critical role in degradation of cellulosic biomass. The gene of cellulose 1,4-β-cellobiosidase having 2502 bp was cloned from a thermophilic bacterium *Caldicellulosiruptor bescii* and overexpressed in mesophilic host *Escherichia coli* BL21 CodonPlus (DE3)-RIPL. The Cbcbh gene was initially cloned in pTZ57R/T and sub-cloned in an expression vector pET-21a(+) and propagated in *E. coli* BL21 CodonPlus DE3-(RIPL). From the multi-alignments and structural modelling studies, it has been revealed that Cbcbh of *C. bescii* has some conserved structural loops and folds. 3D-structure of enzyme (CbCbh) revealed that enzyme is multi-modular in nature and had a conserved cellulose binding domain III. The enzyme's catalytic triad consisted of Glu-380, Asp-404 and Glu-166 that is either involved in substrate or metal binding. After induction of recombinant *E. coli* BL21 with 0.5 mM IPTG, the expression of recombinant protein was examined through SDS-Polyacrylamide Gel Electrophoresis and a prominent band of 91.8 kDa was observed. After analyzing the recombinant enzyme's expression in all cellular fractions, CbCbh demonstrated great activity in cell lysate as compared to other. The enzyme showed activity towards various substrates (e.g. 2% Beech wood xylan, 1% avicel and 4 mg/mL pNPC) and processively released cellobiose from cellulosic substrates. However, CbCbh displayed maximum enzyme activity (46 U/mL/min) with p-nitrophenyl β-D-cellobioside (pNPC) as substrate at 65°C and pH 6.0. All of these important characteristics make CbCbh a viable candidate for biotechnological and industrial applications.