Abstract

A thermostable cellulolytic gene CenC (3,675 bp) encoded 1224 amino acid residues belonging to glycosyl hydrolase family 9, cloned from *Clostridium thermocellum* (ATCC 27405/DSM6725) and over-expressedin mesophilic host *Escherichia coli*BL21CodonPlus using pET21(a)+ expression vector. Amplified gene is initially cloned in pTZ57R/T vector and sub-cloned in pET-21(a)+ vector and transformed *E. coli* BL21 CodonPlus with recombinant vector (pET/CenC). The recombinant CenC is a multi-domain processive endoglucanase, having four binding domains and a catalytic domain. CenC when subjected to SDS-Polyacrylamide Gel Electrophoresis analysisrevealed a prominent band of recombinant protein at 137.11kDa. The optimal activity and expression of CenC was obtained by using reduced concentration of IPTG (0.5mM) and followed by incubation at 37°C with agitation (200rpm) after 6 hours using ZYBM9 medium. An alternative inducer, lactose is used instead of IPTG to induce the expression of recombinant CenC enzyme. With lactose induction best activity was observed in YNG medium. CenC has optimal activity at 70°C and pH 6.0. CenC enzyme displayed great affinity with various substrates, carboxymethyl cellulose, p-nitrophenyl-β-D-cellobioside, avicel, birchwoodxylan, beechwoodxylan, β- glucan barley, laminarin and whatman filter paper and no activity was observed with starch and sucrose. This bifunctional behavior suggests that CenC is a processive endoglucanase which have both exo- and endoglucanase property. Metal ions showed no significant effect on activity of CenC whereas there was a partial inhibition by EDTA (80%), however an increase in the activity up to 125% in the presence of Ca^{2+} - is observed. The enzyme CenC is a potential candidate for the biodegradation of cellulosic biomass for the production of bio-ethanol and various industrial applications.