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CenC, a multidomain thermostable GH9 processive endoglucanase from *Clostridium thermocellum*: Cloning, characterization and saccharification studies

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The growing demands of bioenergy have led to the emphasis on novel cellulases to improve efficiency of biodegradation process of plant biomass. Therefore, a thermostable cellulolytic gene (CenC) with 3,675 bp was cloned from *Clostridium thermocellum* and over-expressed in *Escherichia coli* strain BL21 CodonPlus. It was attested that CenC belongs to glycoside hydrolase family 9 (GH9) with four binding domains, a processive endoglucanase. CenC was purified to homogeneity, producing a single band on SDS-PAGE corresponding to 137.11 kDa, by purification steps of heat treatment combined with ion-exchange chromatography. Purified enzyme displayed optimal activity at pH 6.0 and 70°C. CenC had a half-life of 24 min at 74°C, was stable upto 2 h at 60°C and over a pH range of 5.5-7.5. Enzyme showed high affinity towards various substrates and processively released cellobiose from cellulosic substrates confirmed by using HPLC technique. It efficiently hydrolyzed carboxymethyl cellulose (30 U/mg), β-glucan Barley (94 U/mg); also showed activity towards p-nitrophenyl-β-D-cellobioside (18 U/mg), birchwood xylan (19 U/mg), beechwood xylan (17.5 U/mg), avicel (9 U/mg), whatman filter paper (11 U/mg) and laminarin (3.3 U/mg). CenC exhibited K_m, V_{max}, K_{cat}, V_{max} K_m^{-1} and K_{cat} K_{m-1} of 7.14 mM, 52.4 μmol $mg^{-1}min^{-1}$, 632.85 s^{-1}, 7.34 min^{-1} and 88.63, respectively used CMC as substrate. Recombinant CenC saccharified pretreated wheat straw and bagasse to 5.12% and 7.31%, respectively at pH 7.0 and 45°C after 2 h incubation. Its thermostability, high catalytic efficiency and independence of inhibitors make CenC enzyme an appropriate candidate for industrial applications and cost-effective saccharification process.

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Cloning, characterization and saccharification analysis of GH12 endo-1,4-β-glucanase from *Thermotoga petrophila* in a mesophilic host

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Production of bioethanol has received much attention in recent years and many countries have made large investments in infrastructure, process development, and production facilities. Energy crisis is the leading economic constrain in developed as well as in developing countries. With the exhaustion of non-renewable resources at an exponential rate, the need to develop alternative renewable sources which can be both cost effective, environmental friendly and high in yield is the need of time. Recently, the increasing demand of energy has strongly stimulated the research on conversion of lignocellulosic plant biomass by the action of cellulases enzymes into reducing sugars, for the subsequent production of bioethanol. Endoglucanases are mainly responsible for hydrolyzing the internal glycosidic bond to decrease the length of the cellulose chains. Obtaining efficient and thermostable endoglucanase has become the goal of much research worldwide. Therefore, our research work was focus to search for new resources of endoglucanases, which was thermostable and with high catalytic efficiency. The article focuses on the thermotolerant endo-1,4-β-glucanase gene, of *Thermotoga petrophila* RKU-1, was cloned and over-expressed in *E. coli* strain BL21 CodonPlus for its potential usage for the hydrolysis of lignocellulosic biomass and in different industrial applications. Thermostable endoglucanase can be used simultaneously and directly in the saccharification procedure without a pre-cooling process of biomass. Purified enzyme was optimally active with 530 Umg-1 of specific activity against CMC at pH 6.0 and 95°C, which has exhibited a half- life ($t_{1/2}$) of 6.6 min even at temperature as high as 97°C and stable upto 8 h at 80°C. The recombinant enzyme saccharified pre-treated wheat straw and baggase to 3.32% and 3.2%, respectively after 6 h incubation at 85°C. Its thermostability, resistance to heavy metal ions and high specific activity make endoglucanase a potential and promising candidate for various industrial applications such as in textile industry (in biostoning and biofinishing), in animal feed production, in processing of beer and fruit juice, in biomass hydrolysis (bioethanol production) and in plant oil, detergent, pulp and paper industry.

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