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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 has 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),  $\beta$ -glucan Barley (94 U/mg); also showed activity towards p-nitrophenyl- $\beta$ -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  $\mu\text{mol mg}^{-1}\text{min}^{-1}$ , 632.85  $\text{s}^{-1}$ , 7.34  $\text{min}^{-1}$  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.

### Biography

Ikram-ul-Haq (SI) has completed his Postdoc from Cornell University, New York, USA, and Ph.D. in Industrial Microbiology from University of the Punjab, Lahore, Pakistan. He has been the Dean, Faculty of Science and Technology and founding Director of Institute of Industrial Biotechnology, Government College University (GCU), Lahore. He has successfully completed 16 Projects sponsored by Pakistan Science Foundation, Pakistan Atomic Energy Commission, HEC, GCU & Ministry of Science and Technology while 4 Projects are in process sponsored by MoST, PSF and Pakistan Academy of Sciences. He has published more than 309 papers in journals of international repute, with 1734 citations and has been serving as an editorial board member of reputed Journals

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