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CenC, a multi-domain 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 3,675bp 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 2h 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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Gene interaction, phenotypic and molecular expression of protein in maize

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The genetic architecture and bio-fortification of tryptophan and lysine in maize resulted to protein expression in maize genome. Quality Protein maize (QPM) boosts the nutritional status of man and animal through enrichment of protein. Their roles are increasing in Agriculture to ensure food security and sustainable development of the nation. This study therefore explored the gene action involved in expression of phenotypic traits and molecular profile in QPM maize which included; ILEI-OB, ART/OB/98/SW1, ART/OB/98/SW4, ART/OB/98/SW5 and ART/OB/98/SW6. The damaging effect of *Striga lutea* in endemic zones of Eruwa, South-west of Nigeria had been suppressed due to tolerating genes expressed by ART/OB/98/SW5 and ART/OB/98/SW6. The disease resistance expressed by ART/OB/98/SW6 was also confirmed in in-vitro and field assessment of its interaction with *Aspergillus niger*. The gene interaction and protein expression in ART/OB/98/SW6 and ART/OB/98/SW1 had significantly ($p<0.05$) enhanced drought tolerance and amelioration of mutagenic effects caused by ultraviolet radiation and sodium azide mutagens. Again, the early and maturing genes, morpho-agronomic and yield related traits had been expressed by QPM genotypes. The genomic evaluation of QPM using RAPD marker had also revealed mean total Polymorphic Information Contents (87%), gene diversity (0.88) and allele frequency (9.0) under varied temperature storage levels compared to other maize varieties. The genotype x treatment interaction which was highly significant ($p<0.01$) for most of the phenotypic traits, indicated genetic variation. Therefore, QPM gene should be introgressed for favorable alleles to ensure genetic variation, and thus be encouraged in breeding for maize improvement.

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