INDIAN INSTITUTE OF TECHNOLOGY GUWAHATI
SHORT ABSTRACT OF THESIS

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Thesis Title:
Structure and functional analysis of a recombinant endoglucanase (*At*GH9C-CBM3A-CBM3B) from *Acetivibrio thermocellus* ATCC 27405 and its application in synthesis of nanocellulose-based hydrogel for dye removal

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SHORT ABSTRACT

Cellulose, the most abundant homogenous polysaccharide on Earth, is a renewable and sustainable carbon source. Its efficient degradation requires a synergistic enzyme system known as cellulases. Among these, glycoside hydrolase family 9 (GH9) members exhibit endo-, exo-, or processive endocellulase activity, although the structural basis for this variation remains unclear. This study investigates a novel recombinant GH9 β-1,4-endoglucanase, AtGH9C-CBM3A-CBM3B, from Acetivibrio thermocellus ATCC 27405, focusing on its biochemical properties, the catalytic role of its carbohydrate-binding modules (CBMs), structural conformation, and application in nanocellulose synthesis and dye adsorption. The gene encoding full-length AtGH9C-CBM3A-CBM3B and its truncated variants (AtGH9C-CBM3A, AtGH9C, CBM3A, CBM3B) were cloned, expressed in E. coli BL21(DE3), and purified. AtGH9C-CBM3A-CBM3B displayed maximum activity at 55 °C and pH 7.5, with highest activity toward carboxymethyl cellulose (CMC, 58.8 U/mg), followed by lichenan (44.5 U/mg), β-glucan (36.2 U/mg), and hydroxyethyl cellulose (17.9 U/mg). The catalytic core AtGH9C alone exhibited negligible activity, confirming the essential contribution of CBMs. The enzyme showed pH stability (6.0–9.0), thermal stability up to 60 °C for 90 min, and a Tm of 65 °C. Product analysis revealed generation of cellotetraose and longer oligosaccharides, confirming endo-β-1,4-glucanase activity. Partial recovery of AtGH9C activity by adding CBM3A, CBM3B, or both (47%, 13%, and 50%, respectively) highlighted their role in catalysis and thermostability. Structural modeling revealed an (α/α)_6-barrel fold with a catalytic triad (Asp98, Asp101, Glu489). CD analysis showed 25.2% α-helix, 18.4% β-sheet, and 56.4% random coils, aligning with PSIPRED and SOPMA predictions. MD simulations of the enzyme–cellotetraose complex (200 ns) confirmed enhanced structural stability (RMSD: 1.5 nm vs. 1.8 nm without ligand). RMSF analysis indicated high flexibility in CBM3B, suggesting a lesser role in binding. Docking studies revealed strong binding to cellotetraose (ΔG = –5.05 kcal/mol), with lower affinity for shorter or longer oligosaccharides. Loop 3 (aa 342–379) appeared to block the non-reducing end of cellulose, facilitating processive cleavage and cellotetraose release. SAXS analysis at 5 mg/mL showed a monodisperse, compact, fist-and-elbow-shaped protein. The zeta potential (–24 mV) confirmed stability without aggregation. The enzyme was used with exoglucanase AtCBH5A (62 kDa, 96.82 U/mg activity) to hydrolyze sugarcane trash cellulose

(SCT), extracted via optimized alkaline pretreatment. Enzymatic nanocellulose (EN-NC) was produced by 6 h hydrolysis using 1.5 mg enzyme/g cellulose. For comparison, TEMPO-oxidized nanocellulose (TO-NC) was also prepared. FTIR and XRD patterns were similar for SCT, EN-NC, and TO-NC, while FESEM revealed morphological differences: SCT showed micron-scale fibers (~10 μm), whereas EN-NC and TO-NC displayed nanoscale structures (10–100 nm), with EN-NC showing narrower fibrils (≤20 nm), indicating higher specificity. EN-NC (0.5 g/50 mL) was dispersed in 2% acetic acid and blended with CMC-Na (1.0 g/50 mL) to form NC–CMC hydrogels at 35 °C. Swelling equilibrium was reached within 6 h. The hydrogel showed a pHpzc of 6.6 and a surface area of 4.81 m²/g (vs. CMC alone: 1.57 m²/g). Adsorption studies revealed 88.53% Congo Red (CR) removal at pH 7.5, 48.75 mg/L, 0.47 g dosage, and 4.75 h. Methylene Blue (MB) removal peaked at 94.67% at pH 6.0, 37.5 mg/L, 0.6 g dosage, and 3.5 h. MB followed the Freundlich isotherm (multilayer adsorption), while CR fit both Freundlich and Langmuir models. Kinetics suggested pseudo-second-order adsorption, driven by chemisorption via hydrogen bonding, electrostatic, and n–π interactions. Regeneration was effective using 0.1 M NaOH with 50% ethanol (CR) or 0.1 M HCl/HNO_3 (MB). These results establish NC–CMC hydrogels as promising, eco-friendly adsorbents for tertiary treatment of dye-laden wastewater.