



INDIAN INSTITUTE OF TECHNOLOGY GUWAHATI
SHORT ABSTRACT OF THESIS

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Programme of Study : Ph.D.

Thesis Title: **Cloning, expression, purification, biochemical, functional and structure characterization of an endoglucanase of family GH5_4 (*RfGH5_4*) from *Ruminococcus flavefaciens* FD-1 v3 and its application in lignocellulosic biomass conversion.**

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SHORT ABSTRACT

The gene encoding endoglucanase, *RfGH5_4* was cloned from bacterium *R. flavefaciens* FD-1 v3 and purified showing approx. 41 kDa of molecular mass. *RfGH5_4* had maximum enzyme activity against barley β -D-glucan (665 U/mg) followed by CMC-Na (450 U/mg), lichenan (393 U/mg), xyloglucan (343 U/mg), HEC (333 U/mg) and konjac glucomannan (285 U/mg) at optimum 55°C and pH 5.5 of 20 mM citrate phosphate buffer. It was stable in pH range, 5.0-8.0 and between, 5°C-45°C. *RfGH5_4* had high V_{max} and low K_m against β -D-glucan (689 U/mg and 0.54 mg/mL) and CMC-Na (525 U/mg and K_m of 0.58 mg/mL). 10 mM K^+ increased in the enzyme activity of *RfGH5_4* by 50% possibly suggesting interactions between substrate and the active-site of *RfGH5_4*. The time-dependent TLC analysis of CMC-Na hydrolysates by *RfGH5_4* showed the presence of cellotriose, cellotetraose and other higher oligosaccharides which demonstrated its *endo*-acting mode. TLC, HPLC and MALDI-TOF MS analyses of hydrolysates of *RfGH5_4* treated various polysaccharides showed oligosaccharides (DP2-DP12) confirming its *endo*-mode and multi-ligand activity on soluble substrates whereas cellobiose and cellotriose releasing processive activity on amorphous cellulose. The homology modeling analysis showed the $(\beta/\alpha)_8$ -TIM barrel structure of endoglucanase, *RfGH5_4*. The multiple sequence alignment of *RfGH5_4* showed Glu168 and Glu292 as the catalytic residues. Trp58, Arg80, His122, Asn167, Glu168, Trp179, Ala243, Tyr245, Tyr248, Glu292, Trp325 and His340 are its conserved residues. The CD analysis for secondary structure of *RfGH5_4* displayed 40.8% α -helices, 13.8% β -strands and 45% random coils as deduced by CD analysis as well as PSIPRED and SOPMA servers. Molecular dynamic simulation of *RfGH5_4* structure up to 100 ns at 27°C revealed that it retained a stable conformation as an independent molecule as well as a *Rf*-Cellopentaose complex. The RMSD of *RfGH5_4*-cellopentaose complex (0.55 nm) was lower than that of *RfGH5_4* (0.71 nm) which suggested its compatibility and compactness with the cellulosic ligands. The molecular docking studies of *RfGH5_4* with cellulosic ligands

and hemicellulosic ligands showed strong affinity with cellotetraose, cellopentaose, cellohexaose and cellodecaose, branched xyloglucan (XLLG) and glucomannan oligosaccharides. The open groove of active-site of *RfGH5_4* was responsible for the multifunctionality. The barrier-like conformation of loop L2, L3 and L4 containing amino acid Trp58 at +2 subsite with cellopentaose and cellotetraose imparted the processive behaviour to *RfGH5_4* by which it can consistently hydrolyse cellooligosaccharides into cellotriose and cellobiose. SAXS and DLS analysis showed monodispersed state of *RfGH5_4* at 2.5 mg/mL concentration and the rattle-toy shape in the solution form. *RfGH5_4* deconstructed the NaOH pre-treated lignocellulosic biomasses (Cotton Main Stalk, ptCMS, Cotton Small Branches, ptCSB), Sugarcane Bagasse, ptSBG), *Sorghum durra* stalk, ptSDR), Finger Millet Stalk, ptFMS and Maize Leaves, ptMZL). The maximum Total Reducing Sugar, TRS (mg/g) was observed from ptSDR (72) followed by ptFMS (62), ptSBG (38) and ptCSB (27) in a 48 h saccharification releasing cellooligosaccharides of DP2-DP11 as confirmed by TLC and MALDI-TOF MS. Moreover, synergy of endoglucanase, *RfGH5_4* was also established with cellobiohydrolase, *CtCBH5A* and β -glucosidase, *CtGH1* from *Clostridium thermocellum* (50:50:100 U/g) demonstrating the saccharification of ptSDR, yielding 62.0 mg/g_{ptSDR} of TRS and 28.8 mg/g_{ptSDR} of D-glucose which was further visualised through TLC analysis. These results showed that cocktail, *RfGH5_4*+*CtCBH5A*+*CtGH1* is capable of hydrolysing delignified lignocellulosic biomasses as a potential cellulase toolbox for bioethanol production.

List of Publications

(From the Thesis)

Parmeshwar Vitthal Gavande, K. Kumar, J. Ahmed, A. Goyal, Highly efficient, processive and multifunctional recombinant endoglucanase *RfGH5_4* from *Ruminococcus flavefaciens* FD-1 v3 for recycling lignocellulosic plant biomasses, *Int. J. Biol. Macromol.* 209 (2022a) 801–813 <https://doi.org/10.1016/j.ijbiomac.2022.04.059> 4th April 2022. (JIF 8.0)

Parmeshwar Vitthal Gavande, P. Nath, K. Kumar, N. Ahmed, C.M.G.A. Fontes, A. Goyal, Multifunctionality and mechanism of processivity of family GH5 endoglucanase, *RfGH5_4* from *Ruminococcus flavefaciens* on lignocellulosic polymers, *Int. J. Biol. Macromol.* 224 (2022b) 1395–1411. <https://doi.org/10.1016/j.ijbiomac.2022.10.227> 26th Oct 2022. (JIF 8.0).