



INDIAN INSTITUTE OF TECHNOLOGY GUWAHATI
SHORT ABSTRACT OF THESIS

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Programme of Study : Ph.D.

Thesis Title: Cloning, expression, purification, structure and functional Characterization of Rhamnogalacturonan Lyase (CtRGL) from family 11 Polysaccharide Lyase (PL11) and associated Carbohydrate Binding Module 35 (CBM35) from *Clostridium thermocellum* ATCC 27405

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SHORT ABSTRACT

Pectin is an important component of plant cell wall and is composed of Rhamnogalacturonan I (RG I), Rhamnogalacturonan II and Homogalacturonan. Pectin degrading enzymes such as rhamnogalacturonan lyase are industrially important. Full-length rhamnogalacturonan lyase (*CtRGLf*) from *Clostridium thermocellum* and its truncated derivatives *CtRGL* (catalytic module), *Rgl*-CBM35 (carbohydrate binding module) were cloned, expressed, functionally and structurally characterized. *CtRGLf* and *CtRGL* were found to be alkaline, thermophilic, calcium dependent enzymes with maximum activity against soybean rhamnogalacturonan I. The time dependent TLC analysis showed that *CtRGLf* and *CtRGL* are endo acting enzymes. Modelling and docking studies showed that *CtRGL* possesses a β -propeller fold and its active site is able to accommodate oligosaccharides of up to five residues (DP5). *Rgl*-CBM35 was observed to bind a wide range of ligands including RG I, arabinan, galactan, glucuronic acid and unsaturated pectic oligosaccharides. Small angle X-ray scattering (SAXS) analysis showed that *Rgl*-CBM35 exists in a dimeric form in presence of Ca^{2+} ions. Site-directed mutagenesis and SAXS analysis established that *Rgl*-CBM35 possesses two ligand-binding sites. *CtRGLf* was explored for application in textile industry. *CtRGLf* was used for bioscouring of cotton fabric and degumming of jute fibers. Treatment of cotton fabric with crude *CtRGLf* enhanced its water absorption capacity, which is highly desirable in textile industry. FESEM images revealed that *CtRGLf* treatment of jute fibres was able to make the fibre surface smooth. Smooth surface of jute fibres facilitates their conversion into yarn. Enzymatic bioscouring and degumming are environment friendly alternatives to chemicals being utilised by textile industry.