



INDIAN INSTITUTE OF TECHNOLOGY GUWAHATI
SHORT ABSTRACT OF THESIS

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Programme of Study : Ph.D.

Thesis Title: **Cloning, expression, purification, structure analysis, biochemical characterization and therapeutic applications of a rhamnogalacturonan acetyl esterase (CtPae12B) from *Acetivibrio thermocellus* ATCC 27405.**

Name of Thesis Supervisor(s) : Prof. Arun Goyal

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SHORT ABSTRACT

The thesis work includes general introduction and review of literature of pectin and pectin degrading enzymes followed by cloning of the gene encoding rhamnogalacturonan acetyl esterase (CtPae12B) from the genome of *Acetivibrio thermocellus*. The protein was expressed and purified as a single band with purification fold 18. Detailed structure analysis of the protein was done by computational methods deciphering its catalytic site, catalytic residues, molecular docking with different suitable ligands and molecular dynamic simulation. The solution structure of the enzyme was characterized by small angle X-ray scattering and dynamic light scattering. The biochemical characterization determining the optimal temperature and pH as well as thermostability and pH stability of CtPae12B was determined. Effect of different metal ions on the specific activity of CtPae12B, its substrate specificity and determination of kinetic parameters were performed. The synergistic action of the enzyme was also performed with two other enzymes pectin methyl esterase, CtPME and rhamnogalacturonan I lyase, CtRGLf. The regioselectivity of the enzyme was performed to check the site-specificity and localization of the enzyme targeting the acetyl groups present in rhamnogalacturonan I polysaccharide. The degree of acetylation and methylation of the substrate potato rhamnogalacturonan I (highest specific activity of enzyme, CtPae12B, 700 U/mg) was quantified by different methods. Finally, the role of CtPae12B was elucidated in enhancing the anti-cancer activity of potato rhamnogalacturonan I oligosaccharides in inhibiting colon-cancer cells HCT-116 and also its action in the preparation of controlled and sustainable colon-targeted drug delivery material.