INDIAN INSTITUTE OF TECHNOLOGY GUWAHATI
SHORT ABSTRACT OF THESIS

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Programme of Study	:	**Ph.D.**

Thesis Title: "**Molecular cloning, expression, purification, biochemical characterization and structure analysis of a novel obligate xylobiohydrolase (*Ac*GH30A) from *Acetivibrio clariflavus* ATCC 19732**"

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

The thesis includes a general introduction and literature review on xylan and xylan-degrading enzymes, followed by the cloning of the gene encoding the xylobiohydrolase (*Ac*GH30A) from the *Acetivibrio clariflavus* genome. This study reports the biochemical and structural characterization of a novel GH30 xylobiohydrolase, *Ac*GH30A, from *Acetivibrio clariflavus*. The gene was cloned in pET28a(+), expressed in *E. coli* BL21(DE3), and purified to homogeneity, revealing a ~58 kDa protein. AlphaFold2 modelling and SAXS analysis showed an α/β/α sandwich fold with Glu175 and Glu268 as catalytic residues. Molecular docking and molecular dynamics simulations confirmed high-affinity binding to xylobiose at subsites −1 and −2, supported by ITC (K_a = 7.83×10^5 M^{-1}). *Ac*GH30A displayed optimal activity at 80 °C, pH 7.0, stability from pH 4-7 and 30-70 °C, a melting temperature of 72 °C and a 21-day half-life at 4 °C. It hydrolysed various xylans exclusively to xylobiose, with highest activity against 4-O-methyl glucuronoxylan (V_{max} = 139 U/mg, K_M = 0.71 mg/ml). Enzymatic degumming of *Boehmeria nivea* (ramie) and *Ananas comosus* (pineapple leaf) fibres using *Ac*GH30A alone or in combination with pectate lyase (*Ct*PL1B) and mannanase (*Rf*GH5_7) showed enhanced weight reduction and fibre smoothness. Dual-enzyme mixtures- *Ac*GH30A + *Ct*PL1B for ramie and *Ct*PL1B + *Rf*GH5_7 for PAL and the ternary mix showed superior performance. The enzymatic treatment yielded improved degumming efficiency and fibre mechanical properties. The thermostability, product specificity, and synergistic potential of *Ac*GH30A highlight its potential for applications in the pulp, paper, and textile industries.