INDIAN INSTITUTE OF TECHNOLOGY GUWAHATI
SHORT ABSTRACT OF THESIS

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Programme of Study : Ph.D.

Thesis Title: Cloning, expression, purification, biochemical, structure characterization and application of α-L-arabinofuranosidase (*Ps*GH43_12) of family 43 glycoside hydrolase from *Pseudopedobactor saltans*

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

Thesis Submitted to the Department/ Center : Department of Biosciences and Bioengineering

Date of completion of Thesis Viva-Voce Exam : 20.10.20

Key words for description of Thesis Work : α-L-Arabinofuranosidase, biochemical and structure charachterization, enhanced saccharification

SHORT ABSTRACT

Hemicellulose is the second most abundant polysaccharide present on earth. The presence of arabinose side chains in hemicellulose such as xylan interact with other polysaccharides and forms complex network resulting in the recalcitrant nature. α-L-Arabinofuranosidases hydrolyses the arabinose side chain and having immense industrial importance. *Ps*GH43_12 is the first α-L-arabinofuranosidase from *Pseudopedobacter saltans* belonging to family 43 glycoside hydrolase cloned and expressed. It showed maximum activity at pH 6.5 and 50°C with Vmax 100.7 U/mg, Km 2.17 mM, and kcat/Km 50.27 s-1 mM-1 against pNP-α-L-arabinofuranoside. *Ps*GH43_12 release α- L-araf substitution at O3 and both O2 and O3 positions and therefore, it was classified as type III α- L-arabinofuranosidase. The 3D structure of *Ps*GH43_12 developed by comparative modeling was compact and stable displaying a 5-bladed β-propeller fold at N-terminal and β-sandwich fold at C- terminal. Molecular docking analysis confirmed the involvement of active site residues, Asp71, Asp180 and Glu247 in the catalysis, which was also confirmed by the site-directed mutagenesis of these residues. SAXS analysis displayed that *Ps*GH43_12 is monomeric and a fully folded state in solution form. Guinier analysis gave the radius of gyration between (Rg) 2.8 ± 0.09 nm at 5 mg/ml protein concentration. *Ps*GH43_12 show synergistic behaviour with xylanase. Soaking in aqueous ammonia pretreated sugarcane bagasse on sequential enzymatic saccharification by *Ct*Xyn11A, *Ps*GH43_12 and *Bo*GH43 gave final TRS yield of 164.7 mg/g of ptd SB, with 73.5% hemicellulose conversion and 69.6% xylan to xylose conversion.