INDIAN INSTITUTE OF TECHNOLOGY GUWAHATI
SHORT ABSTRACT OF THESIS

Name of the Student	:	**MADHULIKA SHRIVASTAVA**
Roll Number	:	**206106027**
Programme of Study	:	**Ph.D.**

Thesis Title: "**Structure and functional insights of a recombinant bifunctional a-L-arabinofuranosidase (*Bo*GH43_35) with endo-xylanase activity from *Bacteroides ovatus* ATCC 8483 and its application in fruit juice clarification and waste peel saccharification"**

Name of Thesis Supervisor(s)	:	**Prof. Arun Goyal**
Thesis Submitted to the Department/ Center	:	**Biosciences and Bioengineering**
Date of completion of Thesis Viva-Voce Exam	:	**14-10-2025**
Key words for description of Thesis Work	:	Xylan, Arabinoxylan, *Bacteroides ovatus*, Type-I α-L-arabinofuranosidase, Molecular docking, Molecular dynamics simulation, Fluorescence spectroscopy, Fruit juice clarification, Enzymatic saccharification.

SHORT ABSTRACT

The thesis includes introduction and literature review on dietary fiber and their sources, including the nutritional value of arabinoxylan, its hydrolyzed products, followed by cloning and expression of the putative α-L-arabinofuranosidase, *Bo*GH43_35, a family 43 and subfamily 35 glycoside hydrolase (GH43_35) from *Bacteroides ovatus* ATCC 8483. This study reports biochemical and structural characterization of novel bifunctional α-L-arabinofuranosidase/endo-β-1,4-xylanase. The gene cloned in pHTP1 expressed in *E. coli* BL21(DE3) was purified to homogeneity, revealing a ~74 kDa protein. *Bo*GH43_35 showed maximum activity against wheat arabinoxylan at 37°C and pH 7.0. *Bo*GH43_35 hydrolyzed wheat arabinoxylan with V_{max} 5.4 $U.mg^{-1}$ and K_M 2.7 $mg.mL^{-1}$. *Bo*GH43_35 hydrolyzed products of wheat arabinoxylan when subjected to TLC and HPLC revealed both α-L-arabinofuranosidase and endo-β-1,4-xylanase activities. NMR displayed that *Bo*GH43_35 removes L-arabinose at O-2 or O-3 position from mono-substituted arabinoxylan thereby categorizing it as Type I α-L-arabinofuranosidase. AlphaFold2 revealed 5-bladed-β-propeller fold of catalytic module followed by two consecutive jellyroll type β-sandwich fold by CBM6A and CBM6B. MD simulated structures of *Bo*GH43_35-arabinose complex and only *Bo*GH43_35 revealed stability of *Bo*GH43_35-arabinose complex. Binding analysis of *Bo*GH43_35 by fluorescence spectroscopy against wheat arabinoxylan showed association constant, K_a of 3.11x102 M^{-1} and presence of two binding sites. *Bo*GH43_35 maximized TRS yield in raw pomegranate and mosambi peels producing significant amounts of reducing sugars, with TRS yields of 66 mg/g raw PP and 60 mg/g raw MP. With continued research and industrial adoption, *Bo*GH43_35 has potential to revolutionize fruit processing and lignocellulosic biomass utilization, contributing to a more sustainable agro-industrial sector.