**Biochemical and *in silico* characterization of a novel xylobiohydrolase (*Cc*GH30A) from thermophilic bacterium *Clostridium clariflavum***

A. Yumnam Robinson Singh1 , B. Abhijeet Thakur1

C. Jebin Ahmed1, D. Carlos M. G. A. Fontes2,3 and E. Arun Goyal1

1 Carbohydrate Enzyme Biotechnology Laboratory, Department of Biosciences and Bioengineering, IIT Guwahati, Guwahati, Assam 781039, India.

2 NZYTech – Genes & Enzymes, Estrada do Paço do Lumiar, Campus do Lumiar, Edifício E - R/C, 1649-038 Lisbon, Portugal

3 CIISA – Faculdade de Medicina Veterinária, Universidade de Lisboa, Avenida da Universidade Técnica, 1300-477 Lisbon, Portugal.

E-mail: *yrsingh@iitg.ac.in*

**Research & Industrial Conclave 2023 "An amalgamation of Academia, Industry & Start-up”**

**"**

**Abstract**

Xylans, the primary component of hemicellulose present in lignocellulosic biomass, are the second most abundant polysaccharides present in the plant kingdom. The breakdown of xylan heterostructure requires the combined action of a consortium of different xylanases with non-identical mode of action. In this study, a novel xylanase *Cc*GH30A belonging to family 30 of glycoside hydrolases from *Clostridium clariflavum* was examined biochemically, and the structure was studied by computational approach to understand its mode of action and the structural basis behind its substrate specificity. *In silico* analysis revealed the active site of the enzyme contains Glu175 and Glu268 as catalytic residues. Specific molecular docking showed the maximum binding affinity of *Cc*GH30A with xylobiose, with polar interactions between the catalytic duo and the ligand. The molecular dynamics simulation of the enzyme docked with xylobiose revealed the structural stability of the complex in solution with Glu175, which act as an acid/base, forming a hydrogen bond with the ligand. The gene encoding a putative endo-β-xylanase (*Cc*GH30A; GenBank accession AEV68404.1) was cloned into the pET28a(+) vector and expressed in *E. coli* BL21(DE3) cells. The optimum pH and temperature were 7 and 80°C, respectively. The enzyme was highly stable in a wide range of pH, 4 to 8 and temperature, 30 to 70°C for 90 min incubation. *Cc*GH30A showed maximum activity against beechwood xylan (89.1 U/mg), followed by birchwood xylan (71.74 U/mg) at optimum conditions. *Cc*GH30A displayed Km, 2.45 mg/ml and Vmax, 108.9 U/mg, against beechwood xylan under optimized conditions. TLC, HPLC and LC-MS analysis of the enzyme hydrolyzed products revealed the sole presence of xylobiose. The small-angle x-ray scattering analysis of *Cc*GH30A at 3 mg/ml showed the globular nature and monodispersity of the enzyme molecules. Dynamic light scattering further confirmed the monodispersity of the enzyme in the solution. This thermostable xylobiohydrolase may find potential applications in detergents, food, and fermentation industries.

**Student Academic Board (SAB), Indian Institute of Technology Guwahati, Guwahati, Assam, India**

**Keywords:** Xylan, xylobiohydrolase, xylobiose, beechwood xylan, *Clostridium clariflavum*.

