

Structure and functional insights of an exo- acting endo- β -1,4-xylanase from *Acetivibrio clariflavus* and its applications in Bioconversion and Bio-deinking

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Abstract

Acetivibrio clariflavus DSM 19732, a thermophilic and anaerobic bacterium, is a prolific source of glycoside hydrolases, particularly xylanases, encoded within its cellulosomal machinery. Among these, a gene encoding an endo- β -1,4-xylanase of glycoside hydrolase family 30 (GH30) was identified, designated as AcXyn30B_12 was cloned from *A. clariflavus*, expressed in *E. coli* BL21 (DE3) cells and purified by affinity- and size-exclusion chromatography. Purified AcXyn30B_12 expressed as single band, soluble and homogeneous protein of size 74 kDa. It exhibited highest specific activity against partially acetylated birchwood xylan (PABX) at optimal conditions, 70°C and pH 5.5. AcXyn30B_12 was stable with over 80% activity across a broad pH range, 4.5-8.0 and thermostable up to 60°C. Kinetic analysis revealed a $V_{\max}$ of 133.3 U/mg and K_M of 0.9 mg/mL. TLC, HPLC and MALDI-TOF MS analyses of time-dependent hydrolysis of different xylans from corn cob, oat spelt, beechwood, rye arabino- and wheat arabino-xylan and pretreated sugarcane, kans grass and rice straw showed the release of xylo-oligosaccharides (XOS) from xylopentaose to xylobiose and also xylose confirming both endo- and exo-acting mechanisms. This can be harnessed for diverse industrial purposes, including the production of bioethanol, xylitol, prebiotic XOS and other products. 3D model structure of AcXyn30B_12 was built using AlphaFold2 and analysed using computational methods. The catalytic module forms a $(\beta/\alpha)_8$ barrel structure and 9 β -strands forming a 'side- β structure' characteristics of GH30 family. Molecular docking of AcXyn30B_12 with xylotriose ($\Delta G = -11.2$ kcal/mol) confirmed strong affinity. Molecular dynamics (MD) simulations of AcXyn30B_12-xylotriose complex showed reduced RMSD, R_g and SASA values indicating enhanced stability upon ligand binding. Small angle X-ray scattering analysis of AcXyn30B_12 confirmed the polydisperse and elongated structure. Dummy atom model analysis of AcXyn30B_12 revealed monomeric form at 3 mg/mL and dimeric form at 5 mg/mL. AcXyn30B_12 was evaluated for deinking of newspaper (NEPW) and notebook paper (NOPW). Under optimized conditions (46.4U/g NEPW, 58.6U/g NOPW; 70°C; 90 min), deinking efficiencies reached 81% and 77%, respectively. AcXyn30B_12 improved paper strength (28.1g/m² NEPW, 14.9g/m² NOPW), brightness (75.3% NEPW, 48.0% NOPW) and lower effluent toxicity than NaOH. FTIR, XRD and FESEM confirmed selective hemicellulose degradation and ink removal establishing AcXyn30B_12 as robust biocatalyst for sustainable paper deinking.

Brief Bio-sketch



PhD, Indian Institute of Technology Kanpur, 1995. Post-doctoral Fellow, University College Dublin at Dublin, Ireland, 1996-98; Ohio State University, USA, 1998-1999; Wayne State University, Detroit, USA, Feb1999 - Sep1999; FMV, Lisbon, Portugal, Sep2000 -Aug2003. He was a recipient of Post-doctoral fellowship from FCT at FMV Lisbon.

He joined as Visiting Associate Professor, IIT Guwahati in Aug 2003. Head, Department of Biotechnology, 2009-2013. He is currently, Professor (HAG, Higher Administrative Grade). In last 23 years, he supervised 40 PhD students, executed over 20 projects (including 3 international) as PI, published over 300 papers and over 600 conference papers. His publications have h-index 57 and i₁₀ index of 213.

He received Distinguished Scientist Award 2024 by International Bioprocessing Association (IBA), at Hong Kong, China. He received BHU Centennial Award for outstanding contributions to Microbial Biotechnology and Malviya Memorial Award-Senior Faculty by Biotech Research Society, India. He received “Excellence in Carbohydrate Research (ECR) Award-2017” by Association of Carbohydrate Chemists and Technologists, India, He received Manjrekar Award from Association of Microbiologists of India.

He is elected Fellow of 9 national societies and associations, prominent being Association of Microbiologists of India, Biotech Research Society of India, National Academy of Biological Sciences and National Academy of Agricultural Sciences.

He is elected Fellow of International Bioprocesses Association (IBA) and International Society of Energy, Environment and Sustainability (ISEES).

He was listed in Top 2% Scientists in 2025, in List sourced from Elsevier and Stanford University USA, in the Main field of Enabling & Strategic Technologies (Subfield: Biotechnology).

He was Listed in Top 5% Scientists in 2025 in the List sourced from Sci Rank Global Registry based on Bibliohgrapphic analyses (scirankglobal.org) (# 188871)