DISCOVERY OF A CE16 ACETYL ESTERASE FROM *THERMOTHELOMYCES THERMOPHILUS* AND ITS ROLE IN BIOMASS DEGRADATION

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ABSTRACT

Hemicellulose constitutes 20-40% of lignocellulosic biomass and it is in contact with both cellulose and lignin via inter-molecular bonds. Xylan, the most abundant type of hemicellulose, requires a number of hemicellulases and debranching enzymes for its biodegradation i.e., xylanases, β-xylosidases, arabinofuranosidases, acetic, ferulic, p-coumaric and glucuronic acid esterases or even lytic polysaccharide mono-oxygenases. Acetyl xylan esterases in particular remove acetyl substitutions from the xylan backbone, enabling xylanase access and thus promoting biodegradation. Acetyl groups form ester bonds at positions 2, 3, or both of the xylopyranose residue (xylp), or at position 4 of the non-reducing end xylp. The discovery of acetyl esterases of the carbohydrate esterase (CE) 16 family of the CAZy database revealed a novel specificity towards 3-*O*-acetylated non-reducing end xylp residues further 2-*O*-substituted by 4-*O*-methyl-glucuronic acid, which is considered as one of the most recalcitrant structures of hemicellulose, since it is resistant to the action of all known acetyl esterases. However, the mode of action of CE16 esterases is not yet fully elucidated.

In the present work, a CE16 coding gene from the fungus *Thermothelomyces thermophilus* has been identified. The recombinant protein *Tt*CE16 was heterologously expressed in *Pichia pastoris* and was purified to homogeneity through metal affinity chromatography. Bioinformatics analysis suggests low similarity of *Tt*CE16 amino acid sequence to other characterized CE16 esterases, implying a wide diversity among this group of enzymes. Biochemical characterization of *Tt*CE16 has been determined, revealing a thermostable esterase active on acetylated xylo-oligosaccharides. Its mode of action has also been examined in comparison with acetyl xylan esterases from families CE2 and CE6. Moreover, synergistic relationships between *Tt*CE16 and xylanases of GH10, GH11 and GH30 families on pretreated biomass have been investigated, examining the potential of the esterase to assist enzymatic hydrolysis. Finally, structural studies have been implemented. *Tt*CE16 has been crystallized in space group $P3_12_1$ and X-ray diffraction data have been collected to 1.9 Å resolution. The structure of *Tt*CE16 is currently under determination, applying the molecular replacement technique. The unique functional characteristics of *Tt*CE16 as novel biocatalyst could provide new insights on enzymatic bioconversion of hemicellulose towards circular bioeconomy.

KEYWORDS: acetyl esterase, biomass degradation, esterase-xylanase synergy

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