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The biotechnological potential of a novel CE16 exo-deacetylase from Thermothelomyces thermophilus

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PURPOSE OF THE ABSTRACT

Biotechnological utilization of hemicellulose is an enticing concept, hindered by the recalcitrance of this biomaterial against biodegradation. Acetic acid substitutions of the main-chain carbohydrates contribute to the recalcitrance of biomass, posing a severe inhibitory effect on a number of hemicellulose-targeting enzymes such as xylanases, β-xylosidases and α-glucuronidases. Glucuronoxylan, in specific, is heavily substituted by acetic acid at positions O-2 and O-3, or even O-4 of the non-reducing-end xylopyranosyl residue (Xylp). The acetyl esters of xylan degraded by carbohydrate esterases (CE) which are grouped in the families CE1-CE7 and CE16. The latter family 16 is a growing group of enzymes, involving acetyl esterases, that exhibit an exceptional diversity regarding substrate specificity, regioselectivity and preference on oligomeric or polymeric substrates. However, further insight into the CE16 family is required for their efficient biotechnological exploitation.

In this study, the acetyl esterase TtCE16B from Thermothelomyces thermophilus was heterologously expressed in Pichia pastoris and purified using immobilized metal affinity chromatography. The esterase was biochemically characterized and its mode of action was determined using monoacetates of 4-nitrophenyl β-D-xylopyranosides and multiply acetylated methyl β-D-xylopyranosides as substrate. The first crystal structure of a CE16 representative was determined, in apo- and product (acetate) bound form to 1.9 and 1.42 Å resolution, respectively. TtCE16B structure was solved by molecular replacement, using an AlphaFold prediction as starting model. Finally, the synergistic effect of TtCE16B activity with a number of hemicellulose-targeting enzymes, such as a CE6 acetylxylan esterase, xylanases of the GH10 and GH30 families and a GH43 β-xylosidase, during hydrolysis of delignified beechwood, was investigated.

TtCE16B is an exo-acting deacetylase that performs optimally on oligosaccharides. The esterase targets the non-reducing-end Xylp, removing acetyl groups from positions O-3 and O-4, given that the other vicinal hydroxyl group is free, while a second acetylation at position O-2 does not obstruct the esterase activity. Catalyzing the O-4-deacetylation TtCE16B exhibits complementary regiospecificity to CE6 esterases, which are unable to deesterify this position. Regarding deconstruction of pretreated beechwood, TtCE16B acts in synergy with the β-xylosidase, by deacetylating the non-reducing-end Xylp of the xylooligomers and thus making them accessible to the exo-glycosidase. A minor synergy was also observed when TtCE16B was combined with the xylanases, although their activity is much more enhanced by the CE6 esterase efficiently deesterifying internal acetylated Xylps. Overall, the presence of TtCE16B resulted in increased hydrolysis efficiency of the pretreated biomass. The discovery of novel biocatalysts with distinctive specificities could become an asset for the design of efficient hemicellulolytic cocktails and further assist in the upcycling of residual biomass.

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FIGURES

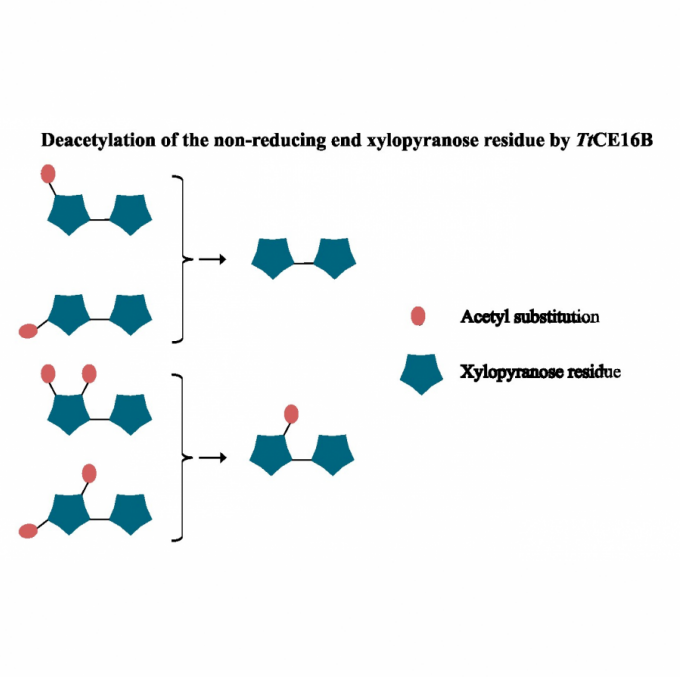

FIGURE 1

Positional specificity of TtCE16B acetyl esterase

TtCE16B preferentially targets acetyl groups from positions O-3 and O-4 of the Xylp residue, given that the other vicinal hydroxyl group is free.

FIGURE 2

KEYWORDS

CE16 acety esterase | Mode of action | Synergies | CE16 crystal structure

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