Assessing the key role of Lytic Polysaccharide Monooxygenases (LPMOs) on the enzyme-mediated preparation of nanocellulose from organosolv pretreated beechwood

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The discovery of lytic polysaccharide monooxygenases (LPMOs) has revolutionized our understanding of the enzymatic conversion of lignocellulosic biomass, particularly in degradation of recalcitrant structures. LPMOs are able to perform the oxidative breakdown of glycosidic linkages of various polysaccharides and exhibit strong synergism with other carbohydrate-acting enzymes (Vaaje-Kolstad et al., 2010). While many aspects of the LPMOs mechanism of action still remain unanswered and their dual ability to act both as monooxygenase and peroxygenase is a highly debated topic (Bissaro et al., 2017), one of the latest findings is the activity on co-polymeric polysaccharide structures present in plant cell walls (Tõlgo et al., 2022). The key role of LPMOs towards the isolation of nanocellulose from natural substrates has been demonstrated (Karnaouri et al., 2022), since these enzymes promote amorphogenesis of the substrate and facilitate the defibrillation process by reducing fiber cohesiveness. LPMOs can be used in combination with cellulases and/or hemicellulases towards the isolation of nanocellulose, thus producing nanostructures which are functionalized with –COOH groups and exhibit good colloidal stability. Since enzyme-mediated nanocellulose preparation processes are gaining increasing attention as they enable elimination of sugar degradation products, inhibitors and toxic compounds that could limit nanocellulose application in food, medical and cosmetic industries (Arantes et al., 2020), elucidating the role of LPMOs is of pivotal importance. The objective of the present work was to elucidate the activity of a novel C1-acting AA9 LPMO from *Thermothelomyces thermophilus*, on cellulosic and hemicellulosic substrates, as well as to evaluate its role on the enzyme-mediated production of nanocellulose from lignocellulosic biomass. The enzyme was heterologously produced in *Pichia pastoris*, purified to its homogeneity and biochemically characterized. Subsequently, it was employed both as a pre- and a post-treatment step alongside with commercially available and in-house produced tailored cocktails of hemicellulases and cellulases in four-step multi-enzymatic processes for the isolation of nanoscale cellulose from OxiOrganosolv pretreated beechwood (Kalogiannis et al., 2020). Nanostructures obtained from each of these green bio-processes were examined for their morphological features and dimensions, crystallinity, colloidal stability and the presence of carboxylate groups. The results demonstrate the formation of well-dispersed nanoscale cellulose in the complete absence of any chemical or mechanical treatment step and verify the importance of efficient hemicellulose removal for the isolation of nanocellulose.

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