EXPANDING THE CATALYTIC POTENTIAL OF A LYTIC POLYSACCHARIDE MONOOXYGENASE FROM THERMOTHELOMYCES THERMOPHILUS: A COMPREHENSIVE STUDY OF POINT MUTATIONS AND STRUCTURAL IMPLICATIONS

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ABSTRACT

This study investigates the impact of point mutations on the functional properties of an AA9 Lytic Polysaccharide Monooxygenase (LPMO)[1], *Tt*LPMO9F, derived from *Thermothelomyces thermophilus*. *Tt*LPMO9F, fused with a carbohydrate-binding module (CBM1), exhibits the ability to oxidize cellulosic compounds with a predominant C4 activity, including phosphoric acid swollen cellulose (PASC) and Avicel, as well as hemicellulosic xyloglucan. The enzyme is notably active on soluble cellooligosaccharides, with higher specific activity towards cellohexaose[2]. However, it exhibits no activity towards polysaccharides such as xylan, chitin, and starch.

Based on available AA9 structural and biochemical data, amino acids potentially implicated in substrate binding were selected as mutation candidates. Point mutations were generated through site-directed mutagenesis and the resulting genes were expressed heterologously in *Pichia pastoris* X33. In total, 8 variants, alongside the wild-type *Tt*LPMO9F, were purified using metal affinity chromatography. Their reaction products on soluble cellooligosaccharides (Degree of Polymerization 5 and 6), as well as polysaccharide substrates such as PASC, Avicel and xyloglucan were analyzed by High-Performance Anion-Exchange Chromatography with Pulsed Amperometric Detection (HPAEC-PAD) chromatography, elucidating the impact of mutations on enzyme function. In order to evaluate these findings at atomic level, crystallographic studies were also pursued. The CBM1 domain was cleaved via papain treatment to facilitate crystal growth, and the X-ray structure of the catalytic domain of *Tt*LPMO9F was subsequently determined at 2.2 A resolution.

Alterations in the rate and product profile of *Tt*LPMO9F variants, combined with structural information, provide valuable insights into the structure-function relationships of LPMOs. Additionally, the study evaluated the synergistic effects, by combining the recombinant enzymes produced in the frame of the present work with commercial enzymatic cocktails for lignocellulosic biomass saccharification.

This comprehensive investigation not only advances our understanding of *Tt*LPMO9F but also provides a solid foundation for the rational design of LPMOs with tailored catalytic properties for industrial applications in biomass conversion and bio-based product synthesis[3].

KEYWORDS: LPMO, mutational studies, biomass saccharification, protein engineering

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