MULTIFUNCTIONAL ENZYME ENGINEERING BY COMPUTATIONAL DESIGN FOR LIGNOCELLULOSIC VALORIZATION

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Biomass- acting enzymes are vital components of biorefinery processes that aim to convert complex, lignocellulosic biomass into fuels, chemicals and materials and therefore, much effort has focused on the improvement of their characteristics (activity, stability, cost of production, etc) as well as on the discovery and development of novel enzymes. Metagenomic approaches revealed that in the Bacteroidetes phylum functionally related genes are often organized in characteristic clusters, known as Polysaccharide Utilization Loci (PUL) reflecting that biomass- acting enzymes act in synergy and that enzyme proximity is important to target complex substrates. In this study we designed a tailored made multifunctional enzyme, combining enzymes isolated from a xylan PUL (1). Computational simulations were performed to define and optimize engineered versions of a multi-domain GH10 endo- xylanase by replacing carbohydrate binding module (CBM) and grafting two new catalytic domains: either a GH43 xylosidase or a CE1 carbohydrate-esterase activities also present in the same PUL. The multifunctional enzymes were then experimentally assessed, demonstrating that chimeric GH10-GH43 had both activities and thus represents a powerful biological tool for hemicellulose deconstruction.

1. Bastien, G., et al. (2013). "Mining for hemicellulases in the fungus-growing termite Pseudacanthotermes militaris using functional metagenomics." Biotechnology for biofuels 6(1): 78