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► **To cite this version:**

Ahmed Khamassi, Thomas Enjalbert, Sandra Pizzut-Serin, Sophie Bozonnet, Estelle Bonnin, et al.. Co-engineering of hemicellulases from a xylan PUL for improved deconstruction of plant biomass. Symposium international Biocatalyse et Biotransformation BIOTRANS, Jun 2023, La Rochelle, France. hal-04747234

HAL Id: hal-04747234

<https://hal.science/hal-04747234v1>

Submitted on 21 Oct 2024

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N°944 / OC / PC

TOPIC(s) : Enzyme discovery and engineering / Artificial enzymes and de-novo enzyme design

Co-engineering of hemicellulases from a xylan PUL for improved deconstruction of plant biomass

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PURPOSE OF THE ABSTRACT

Valorization of Lignocellulosic (LC) biomass is a major economic and environmental challenge. Indeed, LC biomass is a renewable and inexhaustible carbon source on Earth, considered as a key raw material to drive a sustainable economy and reduce our carbon footprint. This biomass is mainly composed of a complex matrix of cellulose and hemicellulose polysaccharides surrounded by lignin, an aromatic polymer [1]. Xylan is the main hemicellulosic polysaccharide in cereals and plays a crucial role in plant cell wall integrity [2]. This polysaccharide is composed of a linear chain of D-xylopyranoside units with β -1,4 linkages that can be substituted by L-arabinose and D-glucuronic acid. Microorganisms have evolved to deconstruct LC and have developed a large set of glycoside hydrolases (GHs), acts in combination with carbohydrate esterases (CE) and lytic polysaccharide monoxygenases (LPMO), all classified in the CAZy database, in order to entangle this complex network and metabolize this carbon source (fig.1) [3]. For the deconstruction of xylan, several main chain depolymerizing GHs, endo-1,4- β -D-xylanases and β -D-xylosidases, are required in combination with debranching enzymes, such as α -L-arabinofuranosidases and/or carbohydrate esterases. GHs have been shown to act in synergy to deconstruct LC and increase the yield of saccharification [4]. However, the molecular determinants that influence enzyme synergy remain unclear [3,4]. In bacteroidetes, these enzymes are often encoded by fine-tuned gene clusters co-regulated for detection, transport and hydrolysis of a dedicated polysaccharide, and called Polysaccharide Utilization Locus (PUL) [5]. Moreover, PUL-encoded GHs were shown to work in synergy and optimize the deconstruction of polysaccharides. In order to investigate the molecular mechanisms involved in enzyme synergy, we “co-engineered” a xylanase and a xylosidase belonging to a xylanolytic PUL [6], meaning we submitted them to a simultaneous cycle of random mutagenesis and screened for pairs exhibiting better arabinoxylan degradation (fig.2). Here, we will present this strategy that allowed the screening of 24 000 mutants and identification of six hits harboring improved activities toward soluble xylan and wheat bran. In order to explain the differences observed in activity and synergy, we will also present the biochemical and structural characterization of the most interesting mutants. This work aims to provide new insights to a better understand enzyme synergy while selecting improved mutants to better valorize lignocellulosic biomass.

FIGURES

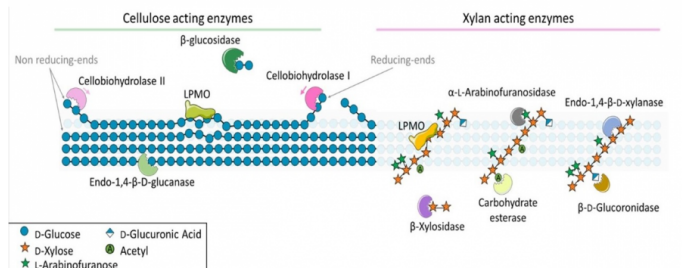


FIGURE 1

Fig. 1 : Schematic representation of enzyme activities degrading cellulose and xylan

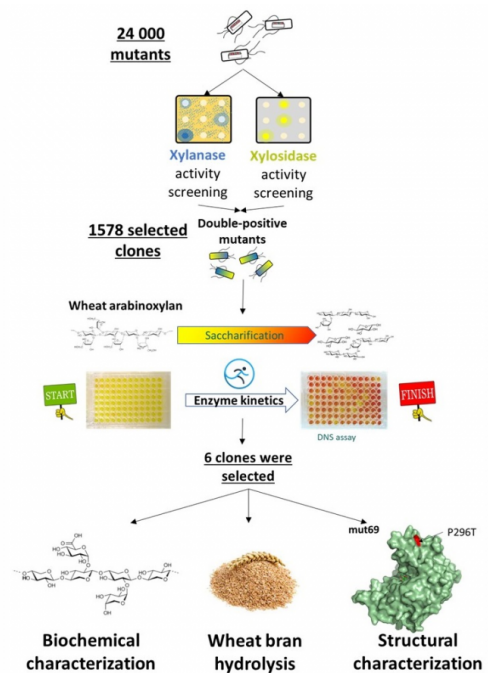


FIGURE 2

Schematic representation of the co-engineering strategy, performed to screen xylanase-xylosidase mutant library

Main steps from the creation of the mutant library to the structure-function characterization of the xylanase-xylosidase positive clones were shown.

KEYWORDS

Xylanase | Xylosidase | Enzyme engineering | Structure-Function Characterization

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