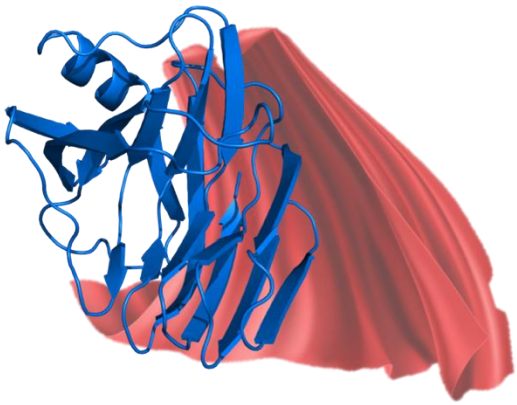
In search of a "superxylanase"

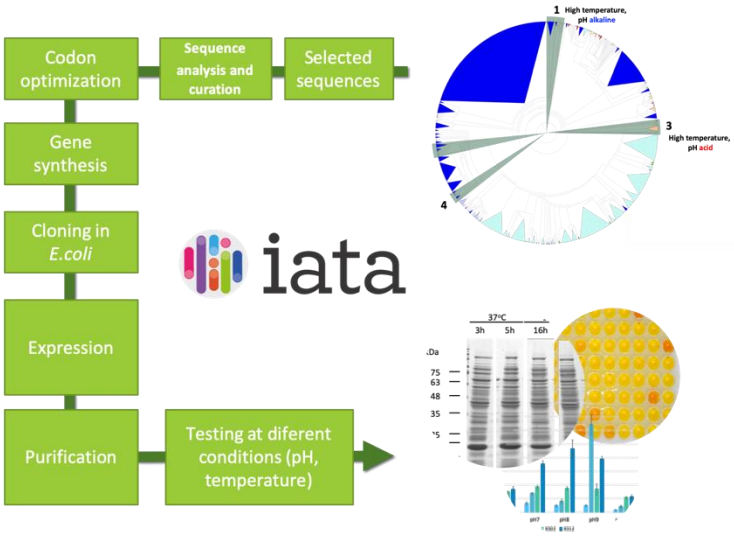


The pulp and paper industry has the challenges of reducing contamination and adding value to byproducts derived from the conversion of wood into paper.

As contribution to that goal, the European Project WoodZymes (www.woodzymes.eu) aims to obtain new enzymes that will increase the value chain from forest biomass to paper, reducing the generation of noxious wastes and finding new uses for currently underutilized byproducts, namely lignin-derived phenols and hemicellulose-derived sugars, in the production of fiberboards, insulation foams and papermaking additives, thus helping to the development of a sustainable bioeconomy in Europe.

A main objective is to find and/or develop enzymes that withstand the extreme conditions of alkaline pH and temperature used in kraft pulp mills. One type of enzymes required for this type of application are xylanases that catalyze the hydrolysis of xylan, the major component of wood hemicelluloses, breaking down the interaction between cellulose and lignin and facilitating the extraction of cellulose.

The Laboratory of Enzyme Structure and Function at IATA-CSIC, led by Dr. Julio Polaina, has recently published a study performed in the frame of WoodZymes project for the identification of extremophilic xylanases through the use of bioinformatic techniques. In the last years, the advance of sequencing techniques has resulted in huge databases, containing an enormous amount of sequences, whose characteristics can be presumed by their similarity to other sequences, but have not been biochemically characterized. Experimental analysis is arduous and time consuming, being a bottleneck in finding enzymes that meet the required conditions. Bioinformatic screening facilitates the task, helping to identify a reduced number of sequences that would be subsequently characterized in the laboratory.



A total of 1306 sequences of GH11, one of the two main families of enzymes acting on carbohydrates in which xylanases are contained, were analyzed bioinformatically. This allowed to group the sequences in a large phylogenetic tree (circular cladogram), and to define the structural composition of each sequence. The position of sequences belonging to known extremophilic microorganisms was marked in the tree, which allowed to define clusters of related sequences from which some could be sampled for detailed analysis.

Synthetic genes encoding the chosen protein sequences were edited to achieve optimal expression in recombinant cultures of *Escherichia coli* used for the production of the enzymes. Assays of enzyme activity were performed under extreme conditions. The analyses showed enzymes capable of working at alkaline pH (pH 9) and temperature of 90 °C. As example of application, the enzymes with best properties were tested for

the digestion of rice straw, a crop residue whose elimination causes serious environmental problems. The treatment with enzymes can provide new uses for rice straw, yielding xylooligosaccharides, sugars with prebiotic properties, that have gained great interest in recent years due to their impact on the beneficial intestinal microbiota.

Read the full paper at:
https://biotechnologyforbiofuels.biomedcentral.com/articles/10.1186/s13068-020-01842-5

Talens-Perales *et al. Biotechnol Biofuels* (2020) 13:198
https://doi.org/10.1186/s13068-020-01842-5

Biotechnology for Biofuels

RESEARCH Open Access

In silico screening and experimental analysis of family GH11 xylanases for applications under conditions of alkaline pH and high temperature

David Talens-Perales[1], Paloma Sánchez-Torres[1], Julia Marín-Navarro[1,2] and Julio Polaina[1*]

Abstract

Background: Xylanases are one of the most extensively used enzymes for biomass digestion. However, in many instances, their use is limited by poor performance under the conditions of pH and temperature required by the industry. Therefore, the search for xylanases able to function efficiently at alkaline pH and high temperature is an important objective for different processes that use lignocellulosic substrates, such as the production of paper pulp and biofuels.

Results: A comprehensive in silico analysis of family GH11 sequences from the CAZY database allowed their phylogenetic classification in a radial cladogram in which sequences of known or presumptive thermophilic and alkalophilic xylanases appeared in three clusters. Eight sequences from these clusters were selected for experimental analysis. The coding DNA was synthesized, cloned and the enzymes were produced in *E. coli*. Some of these showed high xylanolytic activity at pH values > 8.0 and temperature > 80 °C. The best enzymes corresponding to sequences from *Dictyoglomus thermophilum* (Xyn5) and *Thermobifida fusca* (Xyn8). The addition of a carbohydrate-binding module (CBM9) to Xyn5 increased 4 times its activity at 90 °C and pH > 9.0. The combination of Xyn5 and Xyn8 was proved to be efficient for the saccharification of alkali pretreated rice straw, yielding xylose and xylooligosaccharides.

Conclusions: This study provides a fruitful approach for the selection of enzymes with suitable properties from the information contained in extensive databases. We have characterized two xylanases able to hydrolyze xylan with high efficiency at pH > 8.0 and temperature > 80 °C.

Keywords: Carbohydrate-binding domain, Glycoside hydrolase, Rice straw, Xylose, Xylooligosaccharides

WoodZymes project has received funding from the Bio Based Industries Joint Undertaking (JU) under grant agreement No 792070. The JU receives support from the EU's Horizon 2020 research and innovation programme and the Bio Based Industries Consortium. This article reflects only the author's view and the JU is not responsible for any use of the information it contains.

Bio-based Industries Consortium

BBI JU

Horizon 2020
European Union Funding
for Research & Innovation