LIBRARY GENERATION FOR BIOMASS-CONVERSION ENZYMES FROM SOIL METAGENOME

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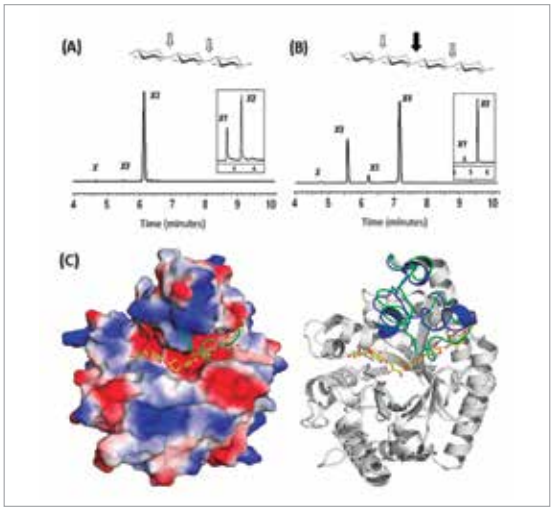

Figure 1. A novel endoxylanase family GH10 (SCXyl) was identified from sugarcane soil metagenome. Along with the description of biochemical characteristics and biotechnological application, SCXyl crystal structure was solved. The ScXyl1 has unusual enzymatic activity against small xylooligosaccharides (A and B) is consistent to the hydrophobic contacts at the +1 subsite and low-binding energies of subsites that are distant from the site of hydrolysis (C). This unusual enzymatic activity is advantageous, because this allows the enzyme to maintain active even in advanced steps of the catalysis, when most of the long xylan chains have been cleaved (Alvarez et al., 2013)

The gradual shift from petroleum to renewable biomass resources is generally seen as an important contribution to the development of a sustainable industrial society and the effective management of green house emissions. Lignocellulosic materials, such as agricultural and forestry residues, are an abundant and low-cost source of stored energy in the biosphere. Thus, biomass conversion into feedstock sugars has moved towards the forefront of the biofuel industry. However, the saccharification of plant biomass is a complicated and lengthy process, mainly due to the inherent recalcitrance and the complex heterogeneity of the polymers comprising plant cell walls. Lignocellulosic biomass must go through an intensive pretreatment step, after which enzymes are used to break down the polysaccharides biomass into simple sugar suitable for fermentation and ethanol production.

Likewise, enzymatic conversion of cellulose and hemicellulose into simple sugar is also a demanding task, where a consortium of enzymes is needed for complete saccharification of these polysaccharides. Aiming at the entire exploitation of the plant cell wall polysaccharides, as an environmentally renewable energy source, an extensive repertoire of hydrolytic enzymes would play a major role for the success of this endeavor towards biofuel production. The objective of our effort is the generation of a toolkit of lignocellulolytic enzymes with a wide range of biotechnological applications, including their use as players for the development of strategies for second generation ethanol production. The prospection of these enzymes will be done from soil metagenome, which contemplates a pioneering strategy towards the prospection of biomass conversion enzymes from microorganisms not conventionally cultivable. Additionally, this study may contribute to the development of the field of bioenergy by improving techniques for characterization of enzymatic hydrolysis and implementing heterologous gene expression in filamentous fungi.

SUMMARY OF RESULTS TO DATE AND PERSPECTIVES

The biotechnology has a continuous demand for novel genes, enzymes and compounds, and natural diversity has been the best supplier for these novel molecules. It is well known that in spite of the vast dataset of enzymes and microbes involved on plant biomass conversion, already described in the literature, it not been discovered yet a super microorganism that is capable of rapidly and efficiently degradation of all components of plant cell wall. Additionally, it is now widely accepted that the application of standard microbiological methods, for the recovery of microorganisms from the environment, has had limited success in providing access to the true extent of microbial diversity. As a consequence, the majority of the microbial genetic diversity (collectively known as metagenome) remains unexploited.

The generation of a library of biomass conversion enzymes, made through heterologous expression, presents a great potential of finding the best cocktails for lignocellulose degradation. Additionally to the wide-ranging industrial applications for these toolkit of hydrolases, the availability of purified cellulolytic and xylanolytic

enzymes shows importance as an analytical tool, not only for deciphering the fine structure of the cell wall architecture, but also for evaluation of required activities for a given pretreatment/enzymatic process for conversion of lignocellulosic biomass to environmentally friendly biofuels.

During the past years, our group has combined high-throughput screening and omics approaches to develop biotechnological routes for production of high-value compounds from plant biomass. We have compiled a collection of enzymes that degrade glycosidic bonds, derived from diverse sources such as soil metagenomes, as fungi, termites, thermophilic prokaryotes and synthetics (artificial) genes. Comprehensive biochemical studies have enabled to assign enzymes to specific biotechnological applications, as well as, molecular dynamics and structural studies have helped to better understand substrate and enzyme interactions at molecular level. Moreover, we have improved fungal systems for protein expression/secretion enabling large-scale production processes.

MAIN PUBLICATIONS

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