LIBRARY GENERATION FOR BIOMASS-CONVERSION ENZYMES FROM SOIL METAGENOME

Fabio Marcio SQUINA
Brazilian Bioethanol Science and Technology Laboratory (CTBE) /
Brazilian Center of Research in Energy and Materials (CNPEM)

Figure 1. Samples were collected from locations where plant biomass degradation is intensively occurring. Our first choice for collection of environmental samples was at a sugarcane field after harvesting. Because covering the soil with straws, it is expected that microbial population involved on lignocellulose degradation is enriched (photo by Luiz P. Jutter - CTBE)

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 micro-organisms 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, so far, 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.

A consortium of microorganisms and enzymes is need for complete saccharification of the plant biomass in natural environment. Our study is focused on the prospection and characterization of the enzymatic system present in difficult-to-culture microbes inhabiting the soil. This study will contribute to the progress of the field of bioenergy by focusing: (1) the generation of library for biomass-conversion enzymes from soil metagenome, (2) improvement of techniques for characterization of enzymatic hydrolysis using capillary electrophoresis and scanning electron microscopy and (3) development of heterologous gene expression in filamentous fungi.

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 celullolytic 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.

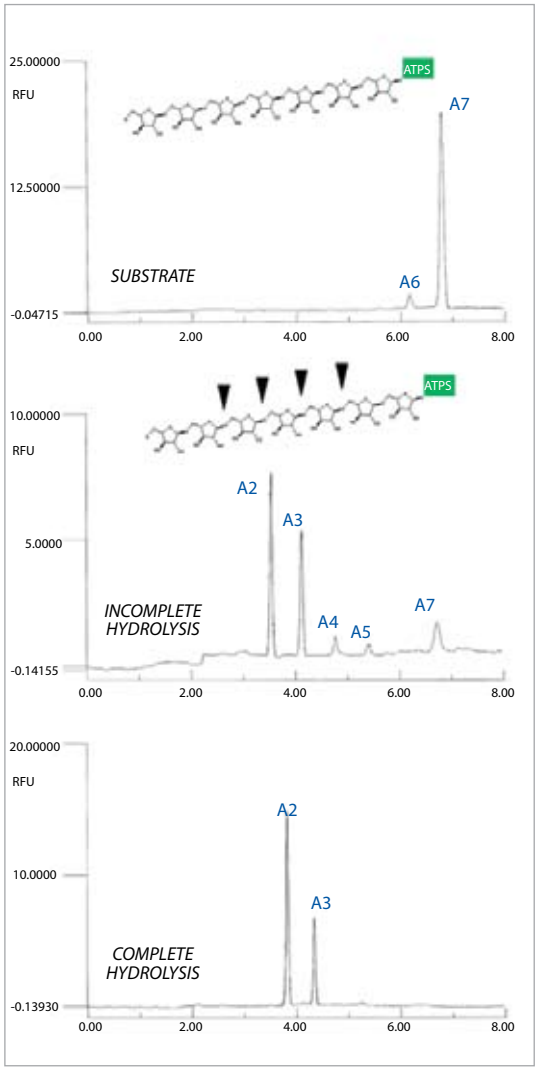

Figure 2. Capillary-zone-electropherogram of APTS-reducing-end-labeled-arabinoheptaose (substrate), intermediary (incomplete) and complete hydrolysis by endo-arabinanase from Thermotoga sp. CZE retention times of arabino oligomers, dimer (A2), trimer (A3) through heptamer (A7), and the predicted mode of operation of endo-arabinanase are depicted

MAIN PUBLICATIONS

Squina FM, Mort AJ, Decker SR, Prade RA. 2009. Xylan decomposition by *Aspergillus clavatus* endo-xylanase. *Protein Expr Purif.* **68**:65-71.

Squina FM, Prade RA, Wang H, Murakami MT. 2009. Expression, purification, crystallization and preliminary crystallographic analysis of an endo-1,5-alpha-L-arabinanase from hyperthermophilic *Thermotoga petrophila*. *Acta Crystallogr Sect F Struct Biol Cryst Commun*. **65**: 902-905.

Squina FM, Prade RA. 2009 Thermohemicellulases for lignocellulosic degradation / US Patent application 61/145.665. Licensed by Edenspace System Corporation for development of transgenic plants for biofuel production.

Fabio Marcio Squina

Laboratório Nacional de Ciência e Tecnologia do Bioetanol (CTBE) / Centro Nacional de Pesquisa em Energia e Materiais (CNPEM)
Rua Giuseppe Máximo Scolfaro, 10.000 – Polo II de Alta Tecnologia – Caixa Postal 6170
CEP 13083-970 – Campinas, SP – Brasil

+55-19-3518-3111
fabio.squina@bioetanol.org.br
www.bioetanol.org.br