

## ***The Lignocellulolytic Metagenome of Sugarcane Bagasse-Degrading-Soil***

Alvarez T. M1., Paixão D. A. A.1, Franco-Cairo, J. P. 1, Vidal R.2., Murakami, M. T. 2, Ruller, R.2, Paes Leme, A.F.2, Pereira, G.A.G.2, Prade R. A.1,3, Squina F.1

1Laboratório Nacional de Ciência e Tecnologia do Bioetanol (CTBE) e 2Laboratório Nacional de Biociências (LNBio) do Centro Nacional de Pesquisa em Energia e Materiais (CNPEM), Campinas, Brazil, 3Department of Microbiology & Molecular Genetics, Oklahoma State University, Stillwater 74078 OK United States.

### **Introduction**

Decades of literature on microbial degradation of plant biomass have resulted in a huge dataset of potential enzymes from an almost as large list of microbes. In spite of that, a microbe that produces an enzyme system capable of rapidly and efficiently degrading all components of plant cell wall has not been discovered yet. Not surprisingly, the commercial enzymatic cocktails, usually derived from fungi, are inefficient in degradation of recalcitrant lignocellulosic biomass, mainly because of the requirement of high enzyme/substrate loads, suggesting that not all the needed activities that enable efficient degradation of biomass are present.

### **Results and Conclusions**

Our working hypothesis is that one organism alone does not encode all the necessary loci that translate into all enzymes needed for lignocellulosic degradation. The focus of our study is *Sugarcane Bagasse-Degrading-Soil (SBDS)*. *Soil* samples were collected in sugar cane field after harvested, non burned plant material, where straws were left on the ground, which is expected to be an environmental community enriched by microorganisms involved in lignocellulosic biomass degradation.

Metagenomes were massively-parallel-454-Roche-sequenced. Comparison of 241,630 Mbp obtained by sequencing of SBDS metagenomic DNA revealed over 500.000 unique ORFs with 225.417 significant hits with proteins deposited in GenBank. The majority of ORFs were from bacteria (89.7%) and archaea (1.34%) and only 0.39% were of eukaryotic-origin. We identified a full repertoire of genes (1.474 different Caazy family members) with significant match to glicosil hydrolases catalytic domain and carbohydrate-binding modules involved in biomass conversion. A subset of 12 putative carbohydrate-active genes are being target for functional analysis. We have been conducting microbial community enrichment experiments on SBDS under semi-anaerobic and forced-aerobic conditions using plant biomass (bagasse) as sole carbon source. Total proteins from the culture filtrate of microbial community were collected and shotgun analyzed by Orbitrap/MS/MS to identify extracellular proteins. Mass spectral data were analyzed in MASCOT using a peptide database created from the predicted ORFs derived from the metagenome sequence. From community enriched systems "culturables" were isolated and gridded onto microtiter dishes and submitted to high throughput lignocellulose breakdown assays. Metagenomic DNA were isolated from enriched communities and target to massively parallel sequencing, as well, meta-expression libraries were prepared in *E. coli*. Bulk IPTG-induced *E. coli* clones were grown on media with cellufloc (insoluble crystalline cellulose), colonies were selected from degradation

halos and inserts sequenced for discovery of novel enzymes involved in lignocellulosic breakdown.

We envision for our study, creation of a comprehensive catalog of proteins, with activities needed to completely degrade natural lignocellulosic substrates. We expect to be able to develop custom enzyme mixtures for complete breakdown of biomass into monosaccharides, as well as aiming at the production of new materials, which could replace petroleum-based routes. This research is supported from FAPESP, grant (08/58037-9) and fellowships for TMA (10/11469-1) and RAP (10-18198-3).

#### Author Publications

Wang, H., Squina F. M., Segato F., Punj, S., Mort, A., Lee, D., Pappan, K. and Prade, R. (2011) High Temperature Enzymatic Cellulose Breakdown, Applied Environmental Microbiology, in press.

Squina F. M. ; Santos, Camila R. ; Navarro, A. ; Oldiges, D ; Leme A. P. ; Ruller, Roberto ; PRADE, R. A. ; Murakami, M. T. (2011). Functional and biophysical characterization of a hyperthermostable GH51 alpha-L-arabinofuranosidase from *Thermotoga petrophila* RKU-1. Biotechnology Letters, v. 33(1), p. 131-137.

Silva, J. C. ; Alvarez, Thabata Maria ; Citadini , A. P. ; de Oliveira, Renata R. ; Oliveira Neto, M.; Ruller, R. ; Prade, R. A. ; Murakami, Mario Tyago ; Squina F. M.(2011). Mode of Operation and Low Resolution Structure of a Multi-Domain and Hyperthermophilic Endo- $\beta$ -1,3-Glucanase from *Thermotoga petrophila*. Biochemical and Biophysical Research Communications (Print), v. 406(4), p. 590-4.

Damasio, A.; Silva, T. ; Almeida, F. B. R. ; Squina F. M. ; Ribeiro, Daniela A. ; Leme A. P. ; Paes Leme, A. F. ; Segato, F. ; Prade, R. A. ; Terenzi, H. F. ; Polisel, M. L. T. M. (2011). Heterologous expression of an *Aspergillus niveus* xylanase GH11 in *Aspergillus nidulans* and its characterization and application. Process Biochemistry, in press.

Squina, Fabio M. ; Santos, Camila R. ; Ribeiro, Daniela A. ; Cota-Silva, Junio ; de Oliveira, Renata R. ; Ruller, Roberto ; Mort, Andrew ; Murakami, Mario T. ; Prade, Rolf A. (2010). Substrate cleavage pattern, biophysical characterization and low-resolution structure of a novel hyperthermostable arabinanase from *Thermotoga petrophila*. Biochemical and Biophysical Research Communications (Print), p. 1-8.

Santos, Camila R. ; Squina F. M. ; Navarro, A. ; Ruller, R. ; Prade, Rolf A. ; Murakami, M. T. (2010) . Cloning, expression, purification, crystallization and preliminary X-ray diffraction studies of the catalytic domain of a hyperthermostable endo- $\beta$ -1,4-D-mannanase from *Thermotoga petrophila* RKU-1. Acta Crystallographica. Section F, v. 1, p. 1078.

Santos, C. R. ; Meza, A. Na.; Hoffmam, Z. B. ; Silva, J. C. ; Alvarez, T. M. ; Ruller, R.; Giesel, G. M. ; Verli, H. ; Squina, F. M. ; Prade, R. A., Murakami, M.T. (2010) Thermal-induced conformational changes in the product release area drive the enzymatic activity of xylanases 10B: Crystal structure, conformational stability and functional characterization of the xylanase 10B from *Thermotoga petrophila* RKU-1. Biochemical and Biophysical Research Communications (Print), v. 403, p. 214-219.

Squina F. M. ; Mort A. J. ; Decker S.R. ; Prade, R. A. (2009). Xylan Decomposition by *Aspergillus clavatus* Endo-Xylanase. Protein Expression and Purification, v. 68, p. 65-71.

Squina F. M. ; Prade, R. A. ; Wang, H. ; Murakami, M. T. (2009). Expression, purification, crystallization and preliminary crystallographic analysis of an endo- $\beta$ -1,5-arabinanase from hyperthermophilic. Acta Crystallographica. Section F, v. 65, p. 902-905.

This document was created with Win2PDF available at <http://www.win2pdf.com>.  
The unregistered version of Win2PDF is for evaluation or non-commercial use only.  
This page will not be added after purchasing Win2PDF.