

FAPESP BIOENERGY PROGRAM

http://bioenfapesp.org



BIOCHEMISTRY

STUDIES OF CELLULAR SIGNALING AND INDUCTION MECHANISMS OF CELLULASES FORMATION BY THE FUNGUS TRICHODERMA REESEI (HYPOCREA JECORINA)

Roberto Nascimento Silva

Ribeirão Preto School of Medicine / University of São Paulo (USP)

FAPESP Process 2010/15683-8 | Term: Jan 2011 to Dec 2014 | Young Investigator

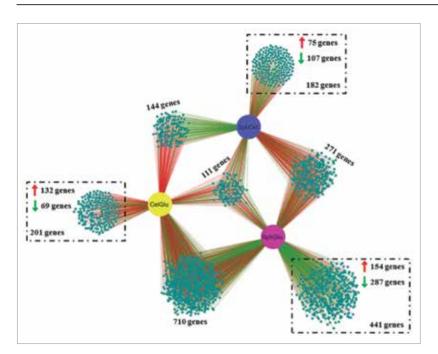


Figure 1. Gene regulatory network (GRN) of 2,060 differentially expressed genes in T. reesei QM9414 in each tested condition. Cellulose versus glucose (CelGlu), sophorose versus cellulose (SphCel) and sophorose versus glucose (SphGlu). Genes are represented as nodes (shown as squares), and interactions are represented as edges (shown as lines, that is, red indicates upregulated interactions and green indicates downregulated interactions), that connect the nodes: 3,385 interactions

The filamentous fungus Trichoderma reesei (Hypocrea jecorina) produces and secretes a large amount of cellulases and hemicellulases that can be used in degradation of biomass components with application in biofuel production. Transcription of the major components of cellulase complex is induced not only by cellulose but also by a variety of disaccharides including lactose, cellobiose, and sophorose and is antagonized by the glucose. However, neither the nature of inducer nor the cellular signaling pathways are totally known. The aim of this project is understand the mechanisms of cellulases formation by the fungus T. reesei as well the mechanism of repression and the cellular signaling pathways involved in these process. As approach, genomic and proteomic techniques were used. cDNA libraries were constructed in different conditions cited above and sequenced using the RNAseq and

expression of the genes differentially expressed were validated by Real Time PCR (RT-qPCR). The secretome and identification of phosphorylated proteins under the conditions cited were analyzed by Differential Gel Electrophoresis 2-D fluorescence (DIGE). With obtained data, a model of global gene expression were constructed using bioinformatics tools what will let a better understanding of gene expression behavior of cellulolytic enzymes produced by *T. reesei* contributing for its application in biofuel industry.



SUMMARY OF RESULTS TO DATE AND PERSPECTIVES

Comparing the parental strain QM9414 transcriptome and mutant strains of *T. reesei* $\Delta xyr1$ and $\Delta cre1$ when grown in presence of the inducers cellulose and sophorose as well as in the presence of the repressor and glucose, new components of the regulatory network were identified such as transcription factors, accessory enzymes, transporters, and the participation of XYR1 and CRE1 were also elucidated. Moreover, new potential targets for these transcription factors in the promoter regions were identified and validated by RNAseg experiments. Furthermore, 2D-DIGE analyses identified 30 proteins exclusive to sophorose and 37 exclusive to cellulose. A correlation of 70.17% was obtained between transcription and secreted protein profiles. We also identified by phosphoproteomic analysis 45 possible targets of protein phosphorylation during sugar cane bagasse hydrolysis. Now we are looking for new functions of transporters, mainly sugar transporters and yet not described transcriptional factors.

We hope that these results contribute to a better understanding of the mechanism of induction and catabolic repression in *T. reesei*, increasing the application of this fungus in different biotechnological areas.

MAIN PUBLICATIONS

Dos Santos Castro L, Pedersoli WR, Antoniêto AC, Steindorff AS, Silva-Rocha R, Martinez-Rossi NM, Rossi A, Brown NA, Goldman GH, Faça VM, Persinoti GF, Silva RN. 2014. Comparative metabolism of cellulose, sophorose and glucose in *Trichoderma reesei* using high-throughput genomic and proteomic analyses. *Biotechnol Biofuels*. 21;7(1):41.

Castro Ldos S, Antoniêto AC, Pedersoli WR, Silva-Rocha R, Persinoti GF, Silva RN. 2014. Expression pattern of cellulolytic and xylanolytic genes regulated by transcriptional factors XYR1 and CRE1 are affected by carbon source in *Trichoderma reesei*. *Gene Expr Patterns*. **14(2)**: 88-95.

Antoniêto AC, dos Santos Castro L, Silva-Rocha R, Persinoti GF, Silva RN. 2014. Defining the genome-wide role of CRE1 during carbon catabolite repression in *Trichoderma reesei* using RNA-Seq analysis. *Fungal Genet Biol.* **73**: 93-103.

Silva-Rocha R, Castro Ldos S, Antoniêto AC, Guazzaroni ME, Persinoti GF, Silva RN. 2014. Deciphering the cis-regulatory elements for XYR1 and CRE1 regulators in *Trichoderma reesei*. *PLoS One*. **18;9(6)**: e99366.

Mello-de-Sousa TM, Rassinger A, Pucher ME, dos Santos Castro L, Persinoti GF, Silva-Rocha R, Poças-Fonseca MJ, Mach RL, Nascimento Silva R, Mach-Aigner AR. 2015. The impact of chromatin remodelling on cellulase expression in *Trichoderma reesei*. *BMC Genomics*. **16**: 588.

Roberto Nascimento Silva

Faculdade de Medicina de Ribeirão Preto Universidade de São Paulo (USP) Departamento de Bioquímica e Imunologia Av. Bandeirantes, 3900 – Monte Alegre CEP 14049-900 – Ribeirão Preto, SP – Brasil

+55-16-3315-3112 rsilva@fmrp.usp.br