

## DEVELOPMENT OF $\beta$ -GLYCOSIDASES DESIGNED TO IMPROVE THE EFFICIENCY OF NONCOMPLEXED CELLULASE SYSTEMS

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FAPESP Process 2008/55914-9 | Term: Jun 2009 to May 2013 | Thematic Project

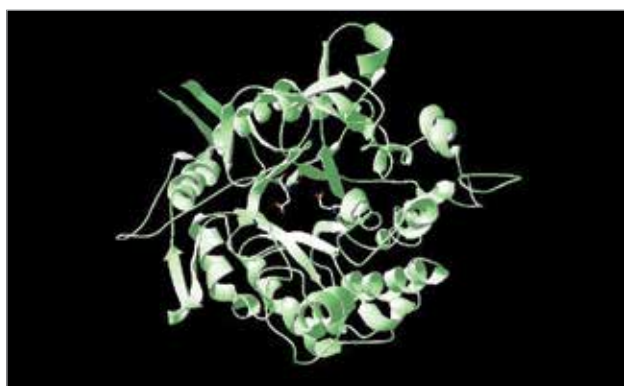


Figure 1. Homology model of Sf $\beta$ gly. Active site residues are represented as sticks

The rate of the enzymatic hydrolysis of cellulose decreases along the reaction time, which represents a drawback for the productivity of this process. One of the factors causing that problem is the cellobiose inhibitory action upon the “cellulases” (endoglucanases and cellobiohydrolases) that catalyze the hydrolysis. This project aims to stabilize the rate of the enzymatic hydrolysis of cellulose by developing  $\beta$ -glycosidases designed to reduce the cellobiose inhibitory effect on the “cellulases”. Such development will be based on the  $\beta$ -glycosidase (cellobiase) from the fall armyworm *Spodoptera frugiperda* (Sf $\beta$ gly) and the “carbohydrate binding domain” of the endoglucanase EngXCA from *Xhantomonas axonopodis pv citri* (CBMXAC).

Firstly, a chimeric protein resulting from the fusion of Sf $\beta$ gly and CBMXAC will be assembled. The targeting of Sf $\beta$ gly to the surface of the cellulose fibers (due the presence of a CBM) could decrease the cellobiose concentration directly in the microenvironment of action of the endoglucanases and cellobiohydrolases. Thus the action of Sf $\beta$ gly-CBM could reduce the cellobiose inhibitory effect and sustain a high activity of endoglucanases and cellobiohydrolases for a longer time.

This project also intends to improve the participation of the  $\beta$ -glycosidases in the cellulose hydrolysis by selecting mutant Sf $\beta$ gly that presents high hydrolytic activity upon cellobiose. Libraries of random mutant Sf $\beta$ gly will be generated and screened based on the ratio of activity upon cellobiose *versus* synthetic substrates. Finally, amino acid residues networks involved in the determination of Sf $\beta$ gly substrate specificity and catalytic activity will be identified by using structural analysis, site-directed mutagenesis studies and enzyme kinetic experiments.

## SUMMARY OF RESULTS TO DATE AND PERSPECTIVES

### Production of the chimeric protein Sfβgly-CBM

The DNA segment coding for the CBM (0.4 kb) of the endoglucanase EngXCA from *X. axonopodis* (AE011689) was amplified taking genomic DNA of that bacteria as template, whereas the segment coding for Sfβgly (AF052729; 1.4 kb) was amplified from a cDNA library of the *S. frugiperda* midgut. Following that, segments coding for CBMXAC and Sfβgly were fused by "overlapping pcr", which generated a product (1.8 kb) coding for the chimeric protein Sfβgly-CBM. This product was cloned into the vector pAE and this construction was introduced in BL21DE3 bacteria. In the next steps Sfβgly-CBM will be produced as recombinant protein and purified by Ni-binding chromatography. The catalytic activity of Sfβgly-CBM will be tested using p-nitrophenyl β-glycosides, whereas its cellulose-binding activity will be verified using avicel.

### Enhancement of the cellobiase activity of Sfβgly

The search for Sfβgly mutants exhibiting high activity upon cellobiose was initiated by constructing libraries by random mutagenesis. A vector pCAL- Sfβgly was used as template according instructions of the kit GeneMorph II EZ Clone. In the next steps the library will be screened using a high-throughput procedure based on the recombinant expression and enzymatic assays in 96-well plates.

### Identification of interaction networks in Sfβgly

In order to identify amino acid residues networks involved in the determination of substrate specificity and catalytic activity the tertiary structure of Sfβgly was represented as a graph and its central hubs were identified. In the next steps 10 of these central hubs will be separately removed by site-directed mutagenesis. The effect of these

deletions on the Sfβgly substrate specificity and catalytic activity by enzyme kinetic experiments.

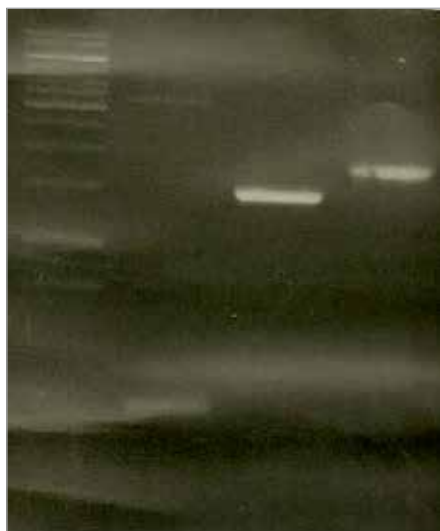


Figure 2. Amplification of the segments coding for CBMXAC (0.4 kb), Sfβgly (1.4 kb) and Sfβgly-CBM (1.8 kb), respectively

## MAIN PUBLICATIONS

Mendonça LMF, Marana SR. 2011. Single mutations outside the active site affect the substrate specificity in a β-glycosidase. *Biochimica et Biophysica Acta – Proteins and Proteomics*. **1814**: 1616-1623.

Mendonça LMF and Marana SR. 2008. The role in the substrate specificity and catalysis of residues forming the substrate aglycone-binding site of a β-glycosidase. *FEBS Journal*. **275**:2536-2547.

Marana SR. 2006. Molecular basis of substrate specificity in family 1 glycoside hydrolases. *IUBMB Life*. **58**:63-73.

Marana SR, Andrade EHP, Ferreira C and Terra WR. 2004. Investigation of the substrate specificity of a β-glycosidase from *Spodoptera frugiperda* using site-directed mutagenesis and bioenergetics analysis. *Eur. J. Biochem*. **271**:4169-4177.

Marana SR, Mendonça LMF, Andrade EHP, Terra WR and Ferreira C. 2003. The role of residues R97 and Y331 in modulating the pH optimum of an insect β-glycosidase. *Eur. J. Biochem*. **270**:4866-4875.

Marana SR, Terra WR and Ferreira C. 2002. The role of Q39 and E451 in the determination of the specificity of an insect beta-glycosidase. *Eur. J. Biochem*. **269**:3705-3714.

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