ANALYSIS OF PROMOTERS FOR THE EXPRESSION OF RECOMBINANT PROTEINS IN TRICHODERMA REESEI

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FAPESP Process 2012/50153-5 | Term: May 2012 to Jul 2014

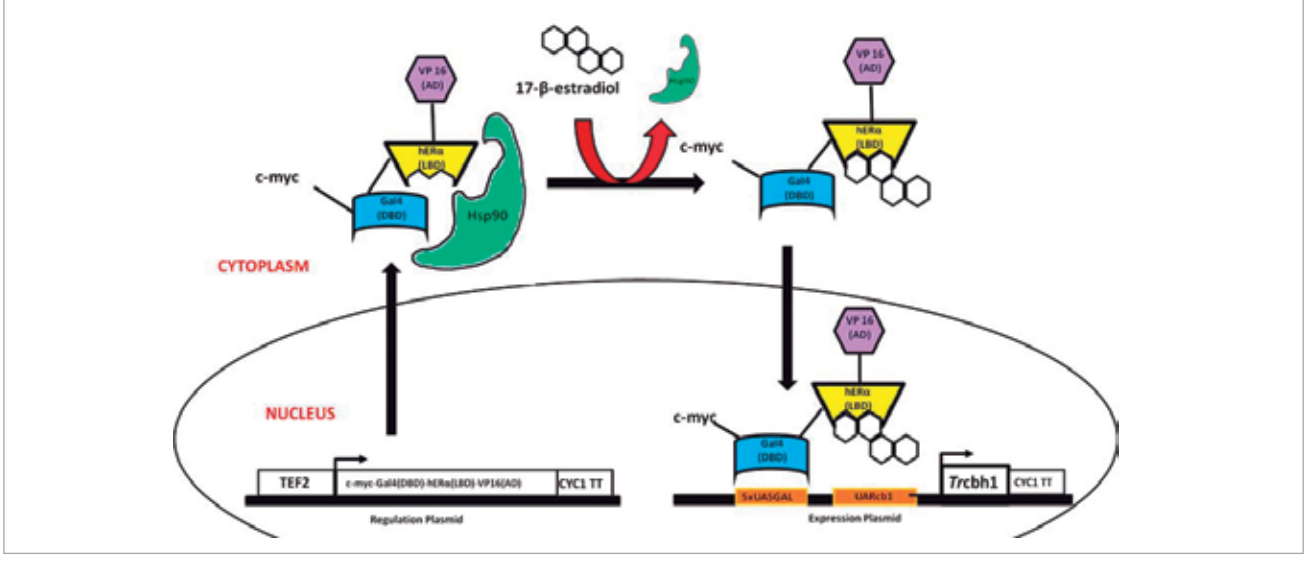

*Figure 1. Induction of the expression of cellobiohydrolase 1 (*cbh1*) gene from* Trichoderma reesei *in the presence of human hormone through the modified* cbh1 *promoter. An estrogen receptor-based gene switch to activate modified Tr*cbh1 *promoter containing five UAS Gal4 motifs from Gal4 gene of* Saccharomyces cerevisiae *was constructed*

The highly efficient secretion machinery of the filamentous fungus *Trichoderma reesei* should be useful for large scale production of homologous or heterologous proteins of industrial interest. We intend to modify a set of metal-induced promoters to allow expression of proteins in large scale using *T. reesei* as host. The design or identification of such promoters would decrease the production costs of enzymes capable of biomass hydrolysis, and that would help the dissemination of biomass use as source of biocombustible.

SUMMARY OF RESULTS TO DATE AND PERSPECTIVES

Our objective is the construction of a system for large scale production of enzymes by means of identification, substitution or modification of *T. reesei* promoters capable of driving a large production and efficient secretion of enzymes involved with the degradation of biomass. The promoter of cellobiohydrolase 1 (*cbh1*) gene of *T. reesei* is induced by cellulose and is strongly repressed by glucose, being commonly used to construct highly efficient heterologous expression vectors. Expression vectors were constructed for *T. reesei* using the following designs: (i) Induction of the expression of *cbh1* and *egl1* genes in the presence of D-sorbitol through the substitution of its native promoter by pyruvate descarboxylase promoter (P*pdc*) and (ii) Induction of the expression of β-galactosidase (*LacZ*) or *cbh1* genes in the presence of metals or human hormone through the modified *cbh1* promoter. *T. reesei* QM9414 mutant strains that express *cbh1* and *egl1* under the control of P*pdc* induced with 200 mM D-sorbitol were analyzed, showing low total protein production (1,3 $g.L^{-1}$ endoglucanase and 0.9 $g.L^{-1}$ celobiohydrolase). Endoglucanase (CMCase) and cellobiohydrolase (Avicelase) activities were 53 IU and 130 IU, respectively, after 24 hours of induction. *Saccharomyces cerevisiae* mutant strains that express *LacZ* were induced by zinc, under the control of *cbh1* promoter modified by inserting Metal Response Elements (MRE) from the *S. cerevisiae zrt1* gene and *S. cerevisiae* strains that express *cbh1* are currently being analyzed for their induction by estradiol, under the control of *cbh1* promoter modified by inserting UASGal elements of GAL4 gene from *S. cerevisiae*. We constructed a set of plasmids with inducible promoters to allow expression of proteins using *T. reesei* as host; however, when using the Ppdc promoter, protein expression is low. Other modified promoters are currently being studied.

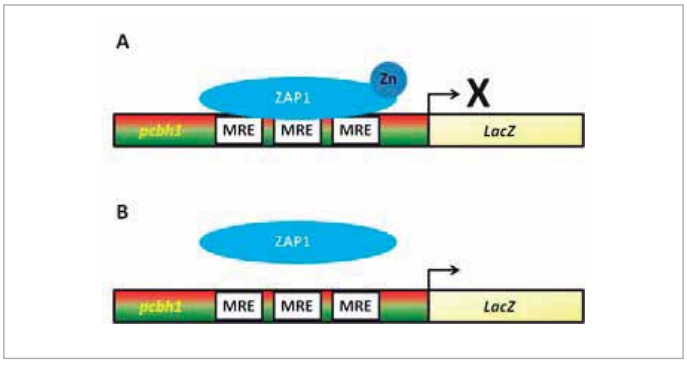

*Figure 2. Induction of the expression of β-galactosidase (*LacZ*) gene in the presence of metals through the modified* cbh1 *promoter from* T. reesei. *An promoter containing three Metal Regulatory Elements (MRE) from zrt1 promoter of* S. cerevisiae, *that express* LacZ *reporter gene under the control of modified Tr*cbh1, *β-galactosidase activity was induced with different Zn concentration. A) High Zn, B) Low Zn*

MAIN PUBLICATIONS

Valencia EY, Chambergo, Felipe S. 2013. Mini-review: Brazilian fungi diversity for biomass degradation. *Fungal Genetics and Biology.* **60**: 9-18.

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Salinas RK, Camilo CM, Tomaselli S, Valencia EY, Farah CS, El-Dorry H, Chambergo FS. 2009. Solution structure of the C-terminal domain of multiprotein bridging factor 1 (MBF1) of *Trichoderma reesei*. *Proteins*. **75(2)**:518-23.

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