A SYSTEM FOR LARGE SCALE PRODUCTION OF RECOMBINANT PROTEINS

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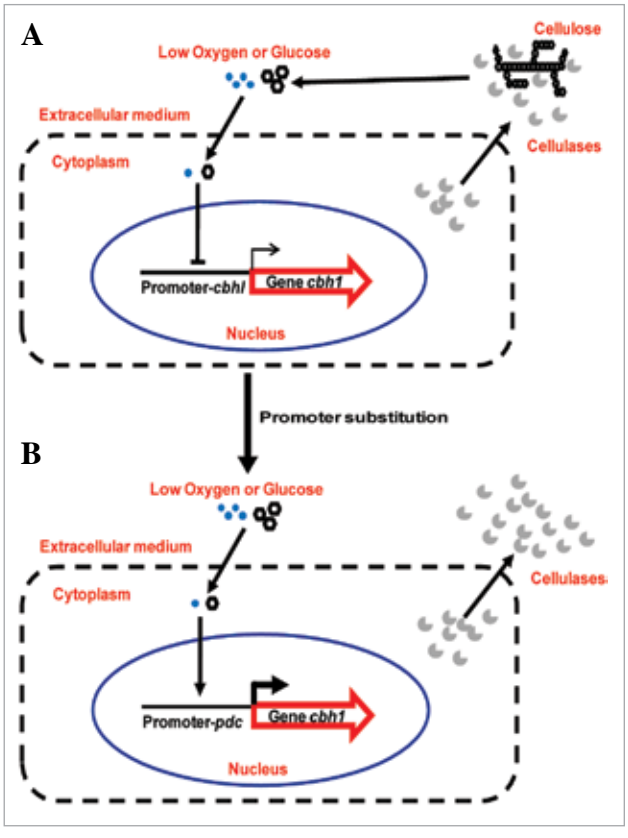

Figure 1. The cellulolytic system of T. reesei *requires induction by cellulose but is repressed by the cellulose degradation product – glucose, or low oxygen tension (A), however, substitution of the promoter (for example, of the cbh1 gene) for the pyruvate decarboxylase (PDC) promoter, which is strongly induced by glucose, or low oxygen tension (B), will be highly efficient to maintain the production of enzymes of industrial interest under repressing conditions*

In 2005, the Brazilian production of sugarcane bagasse summed 106,470 million tons. The utilization of this lignocellulosic residue for ethanol production is viable. It requires, however, a mixture of large amounts of enzymes essential for the hydrolysis of such residue to obtain fermentable sugars. The filamentous fungus Trichoderma reesei possesses an efficient secretory system that could be used in large-scale production of either homologous or heterologous proteins of industrial interest. Our proposal aims the construction of a system for large-scale production of enzymes by means of substitution or modification of *T. reesei* promoters capable of driving a large production and efficient secretion of enzymes involved in the degradation of biomass. The establishment of *T. reesei* mutant strains with prospective industrial use will allow the large-scale production of the necessary enzymes for biomass hydrolysis at a consequent lower cost, favouring the diffusion of biomass utilization as source for biofuels.

SUMMARY OF RESULTS TO DATE AND PERSPECTIVES

In this project we aim to construct a system for large-scale production of enzymes through the genetic manipulation of *T. reesei*. We propose to substitute or modify promoters of this fungus (*Figure 1*) that have the capability of driving a highly efficient secretion of enzymes involved in biomass degradation. This would provide mutant strains with prospective industrial application, which would reduce cost and allow large-scale production of the enzymes required for the hydrolysis of lignocellulosic matter. Vectors bearing homologous or heterologous genes of enzymes involved in biomass degradation (cellulases, exo- and endoglucanases, glycosidases, etc.), under the control of promoters inducible at specific conditions (oxygen tension, concentration of glucose, or specific carbon source, etc.), will be constructed. One potential candidate is the pyruvate decarboxylase (PDC) promoter, which is strongly induced by glucose or low oxygen tension (*Figure 1*) (Chambergo et al., 2002; Bonaccorsi et al., 2006).

MAIN PUBLICATIONS

Bonaccorsi ED, Ferreira AJ, Chambergo FS, Ramos AS, Mantovani MC, Farah JP, Sorio CS, Gombert AK, Tonso A, El-Dorry H. 2006. Transcriptional response of the obligatory aerobe *Trichoderma reesei* to hypoxia and transient anoxia: implications for energy production and survival in the absence of oxygen. *Biochemistry*. **45**:3912-24.

Chambergo FS, Bonaccorsi ED, Ferreira AJ, Ramos AS, Ferreira Junior JR, Abrahao-Neto J, Farah JP, El-Dorry H. 2002. Elucidation of the metabolic fate of glucose in the filamentous fungus *Trichoderma reesei* using expressed sequence tag (EST) analysis and cDNA microarrays. *J Biol Chem*. **277**:13983-8.

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