Topic: Filamentous and Non-filamentous Fungi in Biotechnology and Disease

Title: ***EXPRESSION OF TRICHODERMA REESEI*** **CELLULASES IN THE FUNGUS** ***ASHBYA GOSSYPII***

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Text: **Background**: Due to its natural ability to produce riboflavin (vitamin B2), *Ashbya gossypii* is a widely used microorganism in industry. Additionally, it presents several other interesting features, such as, the smallest known eukaryotic genome, lack of extensive duplication of chromosomal segments and straight forward molecular tools for genetic manipulation. Together, these make it an interesting microorganism, not only for riboflavin production, but also for other applications such as host for recombinant protein production.
The enzymatic degradation of cellulose is of great importance to the carbon cycle of the biosphere, since is the most abundant biopolymer. Bioconversion of cellulose to soluble sugars is catalyzed by a group of enzymes called cellulases.
Objectives:1. Evaluate the potential of *A. gossypii* as a host for recombinant protein production, in particular, cellulases CBHI and EGI from *Trichoderma reesei.* 2. Partially characterize the heterologous cellulases secreted by *A. gossypii*
Methods:1. Expression plasmids for production of *T. reesei* cellulases, namely, CBHI and EGI were used to transform *A. gossypii*. 2. Activity on soluble substrates was determined. 3. The secreted cellulases were partially characterized.
Results: Recombinant EGI was produced by *A. gossypii* in complex, but not in defined medium. Production was similar at 30 and 24°C. Similar amounts of enzyme activity, were produced by *A. gossypii* and *S. cerevisiae*. CBHI activity was not detectable, but the protein could be detected by Western blotting. The cellulases secreted by *A. gossypii* had a different glycosylation pattern than the same proteins secreted by *S. cerevisiae*.
Conclusions: These initial results demonstrate that CBHI and EGI are secreted by recombinant *A. gossypii* into the extracellular medium with similar titers to those secreted by *S. cerevisiae*. Further optimization of the genetic constructions and culture conditions could lead to improved secretion.

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