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High expression levels of *Aspergillus niger* β-galactosidase in *Ashbya gossypii*

Frederico Magalhães [1], Cláudia Dinis [1], Tatiana Q. Aguiar [1], Carla Oliveira [1], Lucília Domingues [1]

[1] Centre of Biological Engineering, Universidade do Minho, Campus de Gualtar 4710-057 Braga, Portugal

Ashbya gossypii is a filamentous Saccharomycete that has been recently considered as a host for the expression of recombinant proteins. However, the expression levels obtained were low, even though similar to those observed in *Saccharomyces cerevisiae* for the same proteins. Here, to further assess the potential of this fungus as a recombinant protein producer, the β-galactosidase from *Aspergillus niger* was successfully expressed and secreted by the *A. gossypii* ATCC10895 strain from 2-micron plasmids carrying the native signal sequence, under the regulation of four different promoters: *A. gossypii* TEF and GPD promoters, and *S. cerevisiae ADH1* and *PGK1* promoters. The native TEF promoter revealed to be the best promoter for the expression of recombinant β-galactosidase in *A. gossypii*, leading to 2 and 7 times more extracellular activity than the GPD promoter and the heterologous promoters, respectively. Furthermore, the levels of recombinant β-galactosidase activity secreted by *A. gossypii* were up to 37 times higher than those secreted by the *S. cerevisiae* CEN.PK 113-7D strain transformed with the same plasmids. In addition, *A. gossypii* expressed 2.5 times more extracellular β-galactosidase activity than the previously reported *A. niger* β-galactosidase producing *S. cerevisiae* NCYC869-A3/pVK1.1 strain. Partial characterization of the recombinant β-galactosidase secreted by *A. gossypii* revealed that this enzyme is extensively glycosylated, as the recombinant β-galactosidase expressed in yeast and the native *A. niger* β-galactosidase. These results highlight the potential of *A. gossypii* as a recombinant protein producer and open new perspectives to further optimize recombinant protein secretion in this fungus.
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Keywords: *Ashbya gossypii*; *Aspergillus niger* β-galactosidase; high recombinant β-galactosidase secretion levels; *A. gossypii* GPD and TEF promoters; *Saccharomyces cerevisiae PGK1* and *ADH1* promoters