

Contributes for the development of *Ashbya gossypii* as a cell factory

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Ashbya gossypii is a filamentous Saccharomycete long known by the scientific and industrial communities, first as a cotton pathogen and subsequently as a riboflavin overproducer. Its industrial relevance combined with its high genetic similarity with *Saccharomyces cerevisiae* promoted the development of a significant molecular and *in silico* toolbox for its genetic engineering. This, together with the increasing knowledge of its genome, transcriptome and metabolism has helped designing effective metabolic engineering strategies for optimizing riboflavin production, and also for developing new *A. gossypii* strains for novel biotechnological applications [1]. Here, we will address our main contributes for the development of *A. gossypii* as a cell factory organism, by presenting an overview of the most representative outputs from our research [referenced in 1, 2].

Envisioning its exploration as a recombinant protein producer, our main efforts focused on the characterization of the *A. gossypii* protein secretory pathway at the genomic, transcriptomic and proteomic levels [2]. Based on experimental observations and on data from omic analyses, a hydrolytic enzyme, invertase, was deduced to be natively secreted by *A. gossypii* and molecularly characterized. The N-glycosylation pattern of the proteins natively secreted by *A. gossypii* was also characterized, as well as the recombinant production by this fungus of secreted proteins from different fungal origins. Among these, the β -galactosidase from *Aspergillus niger* was expressed in *A. gossypii* under the regulation of several native and heterologous promoters, presenting the highest extracellular production levels.

A new molecular tool based on the Cre-loxP recombination system was also developed for generating *A. gossypii* strains free of exogenous selection markers for industrial applications. With this tool, we were able to block the *A. gossypii*'s *de novo* pyrimidine biosynthetic pathway and thus generate a uridine/uracil (*Agura3Δ*) auxotrophic strain, which presents improved riboflavin production under limited uracil/uridine supplementation.

While characterizing the different *A. gossypii* strains (wild and engineered) we have worked with, we have also gathered important information on the physiology of this fungus (e.g., in defined media and in raw substrates such as crude glycerol), which have helped us design strategies to improve its performance, namely improved tolerance to osmotic stress.

These results allowed a considerable advance in the understanding of the biotechnological potential of *A. gossypii* and the production of other metabolites of interest is now being rationally evaluated. To support the rapid development of new strains, more flexible genome editing tools are also being constructed.

References

- [1] Aguiar, TQ, Silva, R, Domingues, L, *Ashbya gossypii* beyond industrial riboflavin production: A historical perspective and emerging biotechnological applications, *Biotechnology Advances* 33, 1774-1786, 2015.
- [2] Aguiar, TQ, Ribeiro, O, Arvas, M, Wiebe, MG, Penttilä, M, Domingues, L, Investigation of protein secretion and secretion stress in *Ashbya gossypii*. *BMC Genomics* 15, 1137, 2014.