Effect of secretory pathway gene overexpression on secretion of a fluorescent reporter protein in Aspergillus nidulans - DTU Orbit (17/08/2019)

**Effect of secretory pathway gene overexpression on secretion of a fluorescent reporter protein in Aspergillus nidulans**

**Background:** The considerable capacity of filamentous fungi for the secretion of proteins is the basis for multi-billion dollar industries producing enzymes and proteins with therapeutic value. The stepwise pathway from translation to secretion is therefore well studied, and genes playing major roles in the process have been identified through transcriptomics. The assignment of function to these genes has been enabled in combination with gene deletion studies. In this work, 14 genes known to play a role in protein secretion in filamentous fungi were overexpressed in *Aspergillus nidulans*. The background strain was a fluorescent reporter secreting mRFP. The overall effect of the overexpressions could thus be easily monitored through fluorescence measurements, while the effects on physiology were determined in batch cultivations and surface growth studies. **Results:** Fourteen protein secretion pathway related genes were overexpressed with a tet-ON promoter in the RFP-secreting reporter strain and macromorphology, physiology and protein secretion were monitored when the secretory genes were induced. Overexpression of several of the chosen genes was shown to cause anomalies on growth, micro- and macro-morphology and protein secretion levels. While several constructs exhibited decreased secretion of the model protein, the overexpression of the Rab GTPase RabD resulted in a 40% increase in secretion in controlled bioreactor cultivations. Fluorescence microscopy revealed alterations of protein localization in some of the constructed strains, giving further insight into potential roles of the investigated genes. **Conclusions:** This study demonstrates the possibility of significantly increasing cellular recombinant protein secretion by targeted overexpression of secretion pathway genes. Some gene targets investigated here, including genes from different compartments of the secretory pathway resulted in no significant change in protein secretion, or in significantly lowered protein titres. As the 14 genes selected in this study were previously shown to be upregulated during protein secretion, our results indicate that increased expression may be a way for the cell to slow down secretion in order to cope with the increased protein load. By constructing a secretion reporter strain, the study demonstrates a robust way to study the secretion pathway in filamentous fungi.

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