Fed-batch production of hydrophobin RodB from Aspergillus fumigatus in host Pichia pastoris

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Fed-batch production of hydrophobin RodB from *Aspergillus fumigatus* in host *Pichia pastoris*

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***Objectives*:** *Aspergillus fumigatus* expresses the hydrophobins RodA and RodB on the surface of its conidia. RodA is known to be important for the pathogenesis of the fungus, but the role of RodB is unknown. The aim was to produce recombinant RodB for further characterication.
***Methods and materials*:** The gene encoding hydrophobin RodB was amplified by RT-PCR from the total RNA isolated from the spores of *A. fumigatus* (AF296 strain). The resulting cDNA was cloned into TOPO vectors, and the inserts were sequenced. The genes were further amplified by PCR and cloned into expression vectors pPICZαA and pPICZB while adding a 6xHis-tag to the C-terminal. The plasmids were linearized, transformed into *P. pastoris* strain X33 and transformants were selected by zeocin resistance. The expression of the RodB gene was first studied in culture flasks in buffered complex methanol medium as protein production was dependent on the methanol-induced AOX1 promoter. Later production was scaled up to a 2 L fed-batch fermentor. Protein production was analyzed by SDS-PAGE, coomassie and silver-stained, as well as western blotting using an anti-his detection antibody. RodB was purified using His-select Nickel Affinity gel. The emulsifying property of rRodB was investigated using olive oil stained with Sudan black suspended in tris-buffer. The stability of oil micelles were studied by light microscopy.
***Results*:** Protein bands of expected size were detected by SDS-PAGE and western blotting in both the fermentation broth and excess foam. Fed-batch production yielded approximately 260 mg/L. rRodB showed good emulsifying properties.
***Conclusion*:** Hydrophobin RodB from *Aspergillus fumigatus* was successfully produced by yeast host *Pichia pastoris* in a fed-batch fermentor with good yields. Functional investigations of the hydrophobin indicated a correctly folded hydrophobin.