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Heterologous expression of hydrophobins RodA and RodB from *Aspergillus fumigatus* in host *Pichia pastoris*

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Introduction

Hydrophobins are small amphipatic proteins present on the spore surface of filamentous fungi. The pathogenic fungus *Aspergillus fumigatus* expresses the hydrophobins RodA and RodB on the surface of its conidia and these may be of importance to the pathogenesis of the fungus.

Aim

Heterologous expression of hydrophobins has proven to be a challenge by past investigators, hence the aim here was to produce pure hydrophobins RodA and RodB in sufficient quantities for further characterization and investigation using the expression host *Pichia pastoris*.

Figures

Figure 1. *Aspergillus fumigatus* colonies (top) and spores (bottom).

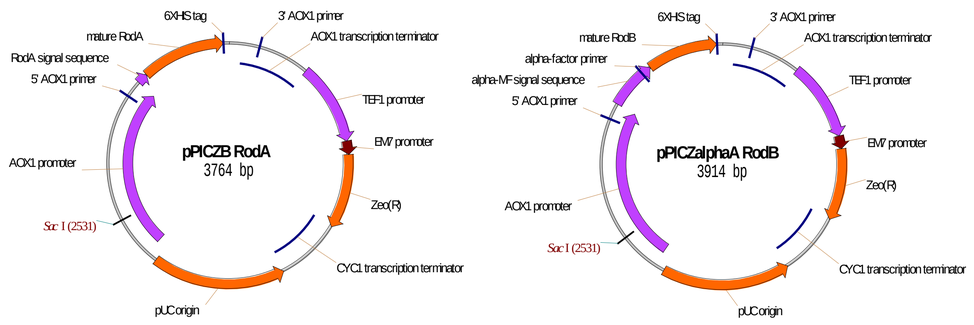

Figure 2. Vectors for hydrophobin expression in *Pichia pastoris.*

Figure 3. Transformants were selected by zeocin resistance and grown in 500 ml shake flasks.

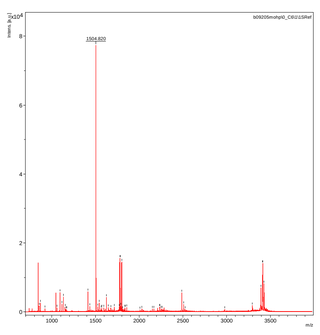

Figure 7. Mass spectrometry spectrum of in gel trypsin digested RodB.

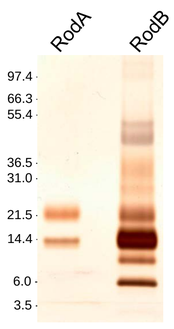

Figure 6. Silver stained SDS-PAGE gel of His-select Nickel Affinity purified recombinant hydrophobins.

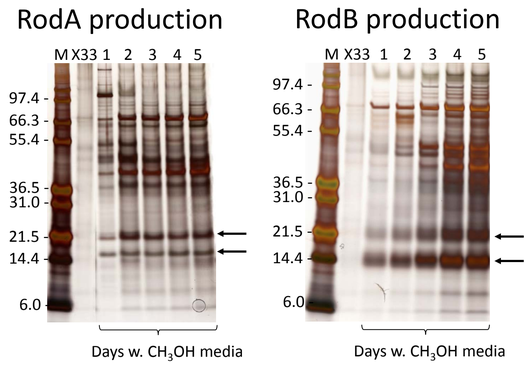

Figure 5. Silver stained SDS-PAGE gels with fermentation broth samples of *P. pastories* alone (X33) and days 1 to 5 of growth on methanol, with is the inducer of hydrophobin expression.

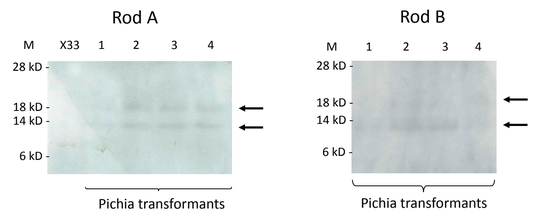

Figure 4. Proteins carrying a 6xhis-tag were identified by western blotting using an anti-his detection antibody. X33 is the untransformed control strain.

Methods

The genes encoding hydrophobins RodA and RodB were amplified by RT-PCR with gene-specific primers from the total RNA isolated from the spores of *A. fumigatus* (AF296 strain). The resulting cDNA was cloned into TOPO vectors using TOPO TA Cloning (Invitrogen), and the inserts were sequenced. The genes were further amplified by PCR to generate overhangs with specific restriction sites and cloned into expression vectors pPICZaA and pPICZB while adding a 6xHis-tag to the C-terminal of both hydrophobins. The pPICZaA vector expresses proteins with the signal sequence of alpha-mating factor from *Saccharomyces cerevisiae* known to work well for protein secretion from *P. pastoris* and the pPICZB plasmids had proteins cloned with their native signal sequences. The plasmids were linearized, transformed into *P. pastoris* strain X33 and transformants were selected by zeocin resistance. The presence of the RodA and RodB genes in the transformants was confirmed by colony PCR. The expression of RodA and RodB genes was induced by growing cells in culture flasks for five days in buffered complex methanol medium as protein production was dependent on the methanol-induced AOX1 promoter.

The protein production was analyzed by SDS-PAGE, coomassie and silver-stained, as well as western blotting using a detection antibody (Penta-His HRP conjugate, Qiagen) and the presence of hydrophobins confirmed by tandem mass spectrometry. Recombinant RodA and RodB were purified using His-select Nickel Affinity gel (Sigma-Aldrich, Saint Louis, MO, USA).

Results

Two hydrophobin genes of *A. fumigatus* were cloned into *P. pastoris* and fermentation broths from 500 ml cultures were analyzed. Proteins of expected size were found by SDS-PAGE and western blotting and confirmed to be RodA and RodB by tandem mass spectrometry (MALDI TOF/TOF). RodA and RodB were purified by His-select Nickel Affinity, and pure yields were 5.4 and 24 mg/L, respectively. We are now working on high cell density fermentations and purification optimization in order to obtain higher protein yields.

Conclusion

Hydrophobins RodA and RodB from *Aspergillus fumigatus* were successfully expressed and secreted in amounts sufficient for further research by the yeast host *Pichia pastoris*.

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