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Heterologous expression of hydrophobins RodA and RodB from
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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.

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 TOP vector 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 pPICZαA and pPICZB while adding a 6xHis-tag to the C-terminal of both hydrophobins. The pPICZαA 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, further amplified by PCR to generate overhangs with specific restriction sites and cloned into EM7 expression vectors. The EM7 vector expresses proteins with a 6xHis-tag and the EM7 promoter.

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.

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.