HETEROLOGOUS PROTEIN EXPRESSION OF MUCOR LUSITANICUS COTH AND HSBA GENES IN PENICILLIUM CHRYSOGENUM AND PICHIA PASTORIS

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Hydrophobic surface binding A proteins (HsbA) belong to the galactomannoprotein family (1) as major components of the fungal cell wall that is released during the growth of fungal hyphae. Our previous studies suggested HsbA proteins influence the biofilm formation and virulence in *Mucor lusitanicus*. Furthermore, CotH3 and CotH4 proteins mediate the process of fungal infection in a cell-wall-dependent manner and play an important role in the pathogenesis (2).

The aim of the present study is to achieve heterologous protein expression of CotH and HsbA proteins in *Penicillium chrysogenum*. Expression plasmids were constructed to able the insertion of the single gene of interest and express the protein inside of the recipient strain. Recombination of the *cotH* and *hsbA* genes into pSK275paf plasmid was performed and transformation procedure was carried out by protoplast formation of *P. chrysogenum*. Transformants were obtained and selected by using minimal media supplemented with phyrithiamine. The heterologous protein was purified and verified with SDS-PAGE and visualised by Coomassie blue and silver staining. The presence of the CotH4 protein was confirmed by MALDI-TOF MS analysis of the fermented broth.

We have started to construct an expression plasmid for heterologous expression of CotH and HsbA proteins in *Pichia pastoris*, using pPICZα vector with an inducible promoter. Proteins were phused with HIS-tag for easier purification. The transformation experiments are ongoing.

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