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Production of a defensin-like antifungal protein NFAP from Neosartorya fischeri in Pichia pastoris and its antifungal activity against filamentous fungal isolates from human infections.

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Abstract

Neosartorya fischeri NRRL 181 isolate secretes a defensin-like antifungal protein (NFAP) which has a remarkable antifungal effect against ascomycetous filamentous fungi. This protein is a promising antifungal agent of biotechnological value; however in spite of the available knowledge of the nature of its 5′-upstream transcriptional regulation elements, the bulk production of NFAP has not been resolved yet. In this study we carried out its heterologous expression in the yeast Pichia pastoris and investigated the growth inhibition effect exerted by the heterologous NFAP (hNFAP) on filamentous fungal isolates from human infections compared with what was caused by the native NFAP. P. pastoris KM71H transformant strain harboring the pPICZαA plasmid with the mature NFAP encoding gene produced the protein. The final yield of the hNFAP was sixfold compared to the NFAP produced by N. fischeri NRRL 181. Based on the signal dispersion of the amide region, it was proven that the hNFAP exists in folded state. The purified hNFAP effectively inhibited the growth of fungal isolates belonging to the Aspergillus and to the Fusarium genus, but all investigated zygomycetous strain proved to be insusceptible. There was no significant difference between the growth inhibition effect exerted by the native and the heterologous NFAP. These data indicated that P. pastoris KM71H can produce the NFAP in an antifungally active folded state. Our results provide a base for further research, e.g., investigation the connection between the protein structure and the antifungal activity using site directed mutagenesis. Copyright © 2013 Elsevier Inc.

KEYWORDS: Antimicrobial susceptibility, Ascomycetes, Clinical fungal isolates, Neosartorya fischeri antifungal protein, Pichia pastoris, Zygomycetes

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Supplement

The increased incidence of severe fungal infections and the fast development of drug resistant filamentous fungi causing mycoses, plant infections or damage of cultural heritages strongly demand for the development of new antifungal strategies. Cysteine-rich antifungal proteins secreted by filamentous Ascomycetes have great potential in these fields. The fact that they (i) have potent antifungal activity against opportunistic human, animal, plant and food-borne pathogenic fungal species [1,2,3], (ii) show no toxic effects on plant and mammalian cells *in vitro* and *in vivo* [2,4,5], (iii) interact synergistically with other antifungal drugs and peptides [3], (iv) are highly stabile [1], and (v) the low cost production render them suitable compounds of commercial preservatives, bio-pesticides and drugs against filamentous fungi [1,2] and offer an alternative, safely applicable solution for the challenges mentioned above.

The *Neosartorya fischeri* antifungal protein (NFAP) (**Fig. 1.**) is a novel representative of the group of cysteine-rich antifungal proteins from Ascomycetes. [6]. NFAP exhibits antifungal activity exclusively against Ascomycetes, it inhibits the germination of spores and the growth of the hyphae causing hyperbranching [6] (**Fig. 2**). Its antifungal activity is dose-dependent: NFAP exhibits growth retardation at sublethal concentrations; and act fungicidal with increasing concentrations [6,7]. NFAP interferes with the organization of the cell wall, destroys the chitin filaments, and triggers apoptotic-necrotic event through ROS accumulation [7].

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