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DISSERTATION SUMMARY

Examination of small antimicrobial proteins and their genetic determinants

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The number of fungal infections has increased continuously over the past years. Infections caused by opportunistic filamentous fungi are especially problematic, because most of the antifungal treatments available have serious side effects and could not be applied without a damage of the host (Vicente et al. 2003). Therefore, there is a substantial demand for new types of compounds with antifugal activity. The defensin-like proteins secreted by some filamentous fungi are interesting from this respect, as they have effective inhibitory potencial both on the hyphal extension and on the germination of the spores. Colllective characteristics of these proteins are the low molecular mass (5.8-6.6 kDa), basic character, 6-8 cysteine residues, and the presence of several disulfide-bonds. Similar proteins have been found and investigated from five fungal species (*Penicillium chrysogenum*, *P. nalgiovense*, *Aspergillus giganteus*, *A. niger*, *Gibberella zeae*); among them only the AFP (*A. giganteus* antifungal protein) from *A. giganteus* and the PAF (*P. chrysogenum* antifungal protein) by *P. chrysogenum* are studied intensively. They have a narrow antimicrobial spectrum, but their specificity are different (Marx 2004). It has been proved that PAF is very effective against opportunistic patogenic zygomycetes (Galgóczy et al. 2005), at the same time it does not harms human cells (e.g. immune cells, nerve cell) *in vitro*. The GAMA (*G. zeae* antimicrobial protein) from *G. zeae* (teleomorf of *Fusarium graminearum*) is a hypothetical protein derived from genomic DNA sequence database. Based on these data, experiments have been carried out to identify new antifungal proteins and their genetic determinants in the genus *Fusarium*.

Fifteen isolates, representing 10 *Fusarium* species (*F. graminearum*, *F. asiaticum*, *F. boothi*, *F. cerealis*, *F. culmorum*, *F. avenaceum*, *F. poae*, *F. polyphialidcium*, *F. sporotrichioides* and *F. pseudograminearum*) have been screened via PCR experiments. Sequences corresponding to hypothetical defensin-like proteins have been found in all isolates. These revealed high similarity to the nucleic acid sequence of the paf gene. Taking into account their nucleic acid and hypothetical protein sequences, 4 types of *Fusarium* antifungal proteins could be differentiated.

The production of antifungal poteins have been optimized. Their biological activities on hyphal growth of *Trichoderma longibrachiatum* and *Mortierella elongata* with an agar diffusion technique have been investigated. Eight of ten showed similar inhibitory effect (like PAF), while the ferment broth of two species (*F. sporotrichioides* and *F. asiaticum*) proved to be inactive in these tests. The supernatant of *F. polyphialidicum* was the most effective in the inhibition of the hyphal extension.

Protein gel electrophoresis revealed the presence of a small protein (approximatly 6.3 kDa) in the eight biological active species. These proteins have been purified further (e.g ultrafiltration). The partially purified protein of *F. polyphilaidicum* maintained its antimicrobial activity. It was supposed, that this 6.3 kDa protein responsible for the antifungal activity, and it was named *Fusarium poliphyalidicum* antifungal protein (FPAP).

The effect of FPAP on germination efficiency of sensitive conidiospores was examined in *T. longibrachiatum*. The conidiospores displayed abnormal, and delayed germination when cultivated in a FPAP-containing medium compared to a control. FPAP-treated conidiospores formed very short, swelled hyphae with multiple branches.

FPAP is a new, small antifungal protein. Further experiments are in progress to clarify its antifungal spectrum, and its effect on plant and mammalian cells.

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