S3

The antifungal protein (AFP) of *Aspergillus giganteus*: Insights into its mode of action and regulation

Vera Meyer, Torsten Theis, Silke Hagen and Ulf Stahl

Proteins with antifungal activity have been isolated from various different organisms ranging from bacteria, plants, insects and amphibians to human beings. The filamentous fungus *Aspergillus giganteus* secretes a basic, low-molecular weight protein with antifungal activities, named antifungal protein (AFP). This protein was found to inhibit the growth of filamentous fungi without affecting the growth of bacteria and yeast. Due to its narrow host range and its high stability, the application of this protein holds promise in the field of plant protection as AFP is highly effective against diverse plant-pathogenic fungi (e.g. *Fusarium* spp.). As the host range of AFP includes opportunistic human pathogens (e.g. *A. fumigatus, A. niger*), the protein is also attractive for use in clinical applications.

Our research group is investigating the target site and the mode of action of the AFP. This should reveal whether the protein is suitable for particular application. We have shown that AFP s growth inhibitory effect is brought about by membrane permeabilization in sensitive fungi. In addition, the accumulation of AFP in distinct areas within the cell wall was only detected for fungi sensitive to AFP. This could be an indication that there is specific binding to some structures within the cell wall. Moreover, we observed that AFP treatment resulted in several membrane alterations of sensitive fungi. Our studies have provided evidence of *in vivo* activity of AFP, as vascular wilt disease of tomato plants caused by *F. oxysporum* was prevented by preincubation of tomato roots with AFP.

Apart from the biotechnological interest in the protein itself, there is also growing interest from the biological point of view. The antifungal activity is the only function of AFP which has been identified to date. As a consequence, it can be speculated that the protein might contribute to an ecological advantage for *A. giganteus* against nutrient competitors and that its expression is therefore triggered under unfavourable growth

conditions. In agreement with this hypothesis, we found out that highest AFP yield is reached during stationary growth, i.e. when nutrients become limited, and that *afp* transcription is enhanced by heat shock, osmotic stress, carbon starvation and by the presence of certain co-cultivants. In addition, it is most interesting that the AFP titre is significantly enhanced under high pH conditions (pH 8), as the pH within soil is mostly alkaline.