APPLICABILITY OF *PENICILLIUM CHRYSOGENUM* ANTIFUNGAL PROTEIN AND ITS RATIONAL DESIGNED VARIANT IN PLANT PROTECTION

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The enormous crop losses worldwide caused by pesticide-resistant pathogenic fungi in pre- and postharvest conditions represent a serious challenge for the agriculture in every year. Application of alternative biopesticides provides a promising basis to overcome this problem. Our previous studies demonstrated that the extracellular, cationic and cysteine-rich antifungal protein PAF from *Penicillium chrysogenum* effectively inhibits the growth of several other filamentous ascomycetes. In the present study we report for the first time the improved efficacy of the PAF variant PAFγ^{opt} against plant pathogenic fungi. In PAFγ^{opt} specific amino acids in the evolutionary conserved antimicrobial gamma (γ)-core motif were substituted to elevate the positive net charge and the hydrophilicity of PAF (Sonderegger et al., Front. Microbiol. 2018, 9, 1655). PAFγ^{opt} effectively inhibited the growth of the plant-pathogens *Cladosporium* and *Fusarium* spp. *in vitro*, but proved to be ineffective against aspergilli. In contrast, *Aspergillus* spp. showed high susceptibility to the wild-type PAF, while cladosporia and fusaria were less susceptible in comparison with PAFγ^{opt}. Double minimal inhibitory concentration (2×MIC) of PAF and PAFγ^{opt} did not affect the viability of *Medicago truncatula* germlings in plant toxicity assay. Furthermore, the root length and the side root number of the plants growing in the presence of PAF and PAFγ^{opt} were not significantly different from the untreated control. *In vitro* plant protection experiments demonstrated that the treatment of *M. truncatula* germlings with 2×MIC PAFγ^{opt} decreased the symptoms of *Fusarium oxysporum* infection. *In vitro* cytotoxicity tests excluded any toxic effects of PAF and PAFγ^{opt} on human keratinocytes, intestinal epithelial cells and leukocytes when applied at 2×MICs; furthermore, haemolytic

activity was not observed at this concentration. Our proof-of-principle experiments promise the successful application of *de novo* rationally designed antifungal proteins in plant protection and the development of novel antifungal strategies for the use in agriculture.

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