Title The peach (*Prunus persica*) defensin *Ppdfnl* displays antimicrobial activity against fungal pathogens through specific lipid binding and membrane permeabilization

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Abstract

Plant defensins are a large fami Iy of small, stable, cationic, cysteine-rich peptides playing a crucial role in the plant innate immunity. For their biochemical features and their broad-range antimicrobial activity, plant defensins are interesting targets for several agro-biotechnology applications. *Ppdfn1* is a gene encoding for a peach (*Prunus persica*) defensin, previoulsy reported to be active against the blue mould *Penicillum expansum.* In this study, we show that *Ppdfnl* is highly expressed in flowers and we investigate on its antifungal properties. The recombinant mature peptide PpDFN1 was expressed in *E. coli* and purified to homogeneity. The activity of recombinant PpDFN1 was tested in vitro against fungal and bacterial plant pathogens; PpDFN1 resulted active against *Botrytis cinerea, Monilinia lena* and *P. expansum,* with IC50 values of 15.1, 9.9, and 1.1 μg/ml, respectively. Treatment of fungal hyphae with FITC fluorescent labelled PpDFNl indicated that the peptide was not internalized by fungal hyphae, but localizes on the external surface of cells. Here, PpDFN1 is capable of membrane destabilization and permeabilization, as demonstrated by SYTOX green fluorescence uptake by the treated mycelia. Using artificial lipid monolayers we showed that PpDFN1 interacts with lipid membranes, supporting indications on lipid binding properties of plant defensins. However, PpDFN1 displayed stronger interaction with monolayers composed merely by lipids extracted from sensitive fungi. In particular, the highest affinity was shown for lipids extracted from *P. expansum,* most sensitive to PpDFN1 action.