

Exploring vertebrate cathelicidins: a new weapon against *Botrytis cinerea*

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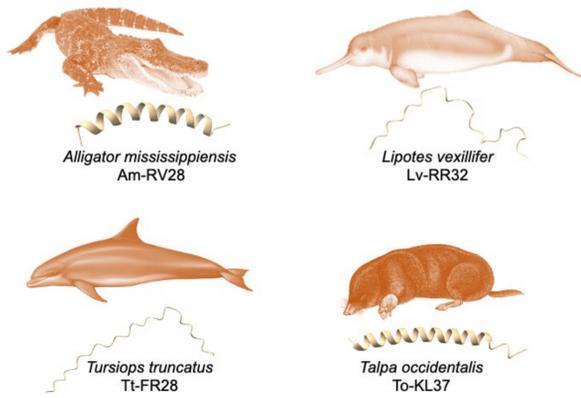
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Introduction

Botrytis cinerea is a fungal phytopathogen that has a significant impact on agriculture worldwide. The predominant approach to botrytis control is the use of synthetic organic fungicides. Finding alternative strategies to control this fungal infection is a major challenge. In this study, we evaluated the efficacy of four peptides from the cathelicidin family in controlling *B. cinerea* infection in vitro and in planta using tomato leaves. This study serves as a basis for further research on the potential of these peptides for crop protection and disease control.

Materials and Methods

Cathelicidin peptides



Name	Sequence	Length	MW	pI	Net charge	GRAVY
Am-RV28	RVTRWLWLLRGGKKAAGWGIRAHNLNRNQ	28	3299.88	12.60	6	-0.429
Lv-RR32	RRIRIRPPRLPRPRPRPWFPFRFPIPRIPGKR	32	4035.95	13.00	12	-1.403
To-KL37	KLFGKVGNNLQKGWQKIKNIGRRIKDFFRNIRPMQEA	37	4429.30	11.60	8	-0.751
Tt-FR28	FRPPYLRPRGRRPRFPFPPIPRIPRIP	28	3391.12	12.40	7	-0.954

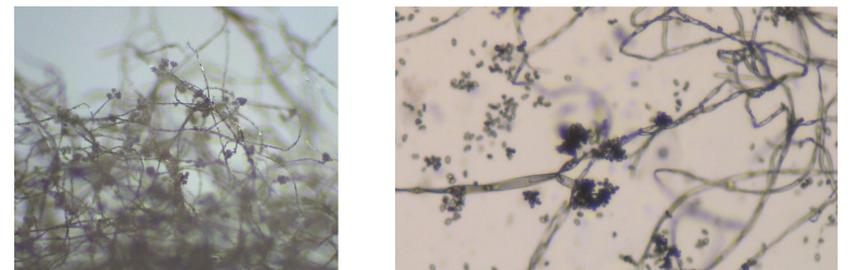
In vitro assays

- Germination of conidia after 4, 6, 8 and 10 h of incubation in contact with the peptide (at 25, 50 and 100 μ M) for 10 hours.
- Plate culture of conidia previously exposed to each of the peptides and concentrations for 24 hours.

In planta assay

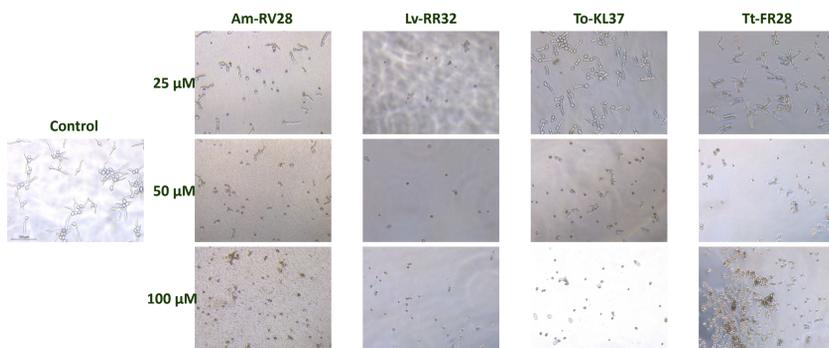
- Infection of tomato leaves with conidia treated or untreated with peptides.

In vitro and in planta assays



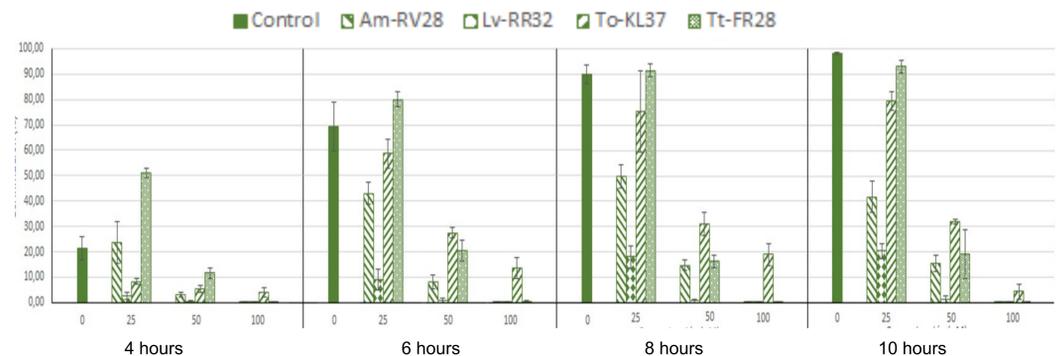
3. Results

Germination of conidia



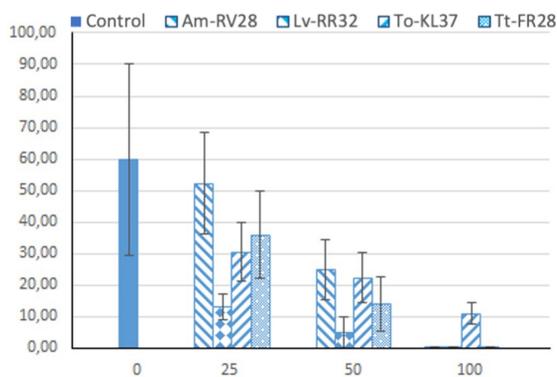
In vitro assays showed that all peptides completely inhibited the germination of conidia at the concentration of 100 μ M. In addition, they also affected hyphal growth at concentrations of 50 and 25 μ M, highlighting the activity of peptides Lv-RR32 and Am-RV28 which showed higher activity at these concentrations.

Percentage of conidia germination



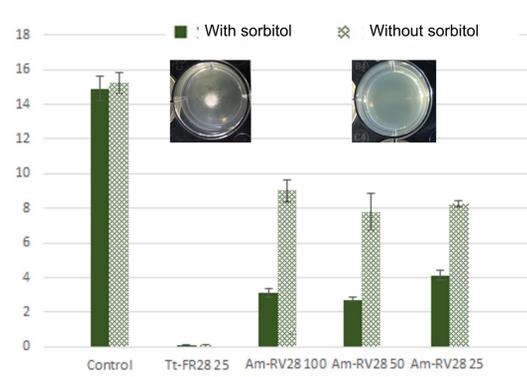
The y-axis represents the percentage of germination. The x-axis represents the peptide concentrations, namely 0 mM, 25 mM, 50 mM, and 100 mM. At a dose of 100 μ M, all peptides showed complete inhibition of conidia germination. The experiments were performed in triplicate.

Hyphae length measurement



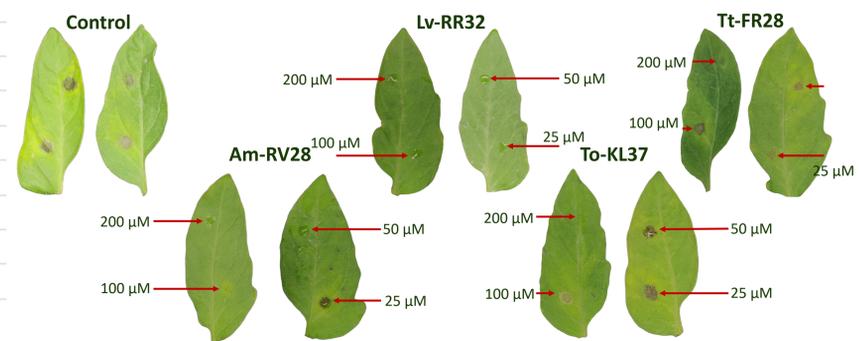
The y-axis represents the percentage of germination. The x-axis represents the peptide concentrations at μ M. After 10 hours all peptides affected hyphal growth at concentrations of 50 and 25 μ M, highlighting the activity of Lv-RR32 and Am-RV28 peptides that showed higher activity at these concentrations.

Lethal effect of peptides on conidia



The osmotic stabilizer sorbitol (1M) was used to study cell wall integrity. Sorbitol impacted on the inhibition zone of the fungus after peptide treatment.

Tomato leaf infection



Results were in agreement with what was seen in the in vitro experiments. Peptides Lv-RR32 and Am-RV28 completely inhibited the infection at the concentration of 100 μ M. On the other hand, the Tt-FR28 peptide reduced the infection at 100 μ M, being completely inhibited at 200 μ M. Finally, the To-KL37 peptide did not inhibit infection, although infection was lower at the maximum concentration tested (200 μ M).

4. Conclusions

The present study provides the first evidence for the inhibitory properties of cathelicidins in an in vitro environment and suggests the potential of cathelicidins as peptide inhibitors to suppress the growth of *Botrytis cinerea* in plants. The in vitro efficacy of several cathelicidins against *B. cinerea* was demonstrated. The potential efficacy of certain peptides in the range of 25 micromolar or perhaps even below is currently under investigation. Vertebrate cathelicidins have the potential to serve as a new source of alternative antifungal chemicals compared to the pesticides already in use. The results presented and the diversity of cathelicidins available provide an opportunity to explore new antifungal agents within this class of compounds.

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