AMP32: a short cyclic peptide as an environmentally friendly alternative to counteract late blight symptoms in crops

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(I) Potato late blight (*P. infestans*) infection, (II) *P.infestans* sporangia, (III) and mycelium.

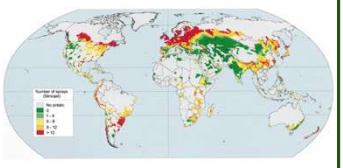
Abstract

Oomycetes, e.g. *Phytophthora infestans*, a widespread pathogen that causes late blight and affects most *Solanaceae* species, cause crop losses of several billion USD every year.

Oomycetes life cycle consist in the production of sexual oospores and asexual zoospores, this event occurs numerous times during a single vegetative season. Defence against fungal plant pathogens is mainly based on the use of chemical fungicides[1] , which give rise to great concerns for risks associated with human health, environmental toxicity, or fast development ofresistance in target pathogens. Among those, several compounds have been banned or included in a list of candidates for substitution by the EU Commission, thus novel and sustainable molecules with low environmental impact are needed. Our group identified, through a Yeast Two-Hybrid-based combinatorial library of cyclic peptides (CYCLIC), an antimicrobial peptide (AMP32) able to bind a bait (i.e., target) of interest[2]. This approach aims to increase the specificity toward the targeted protein and, as consequence, toward the desired pathogen, thus reducing the risks of negative effects on other organisms. In this work, the glycosyltransferase domain of the *P. infestans* Cellulose synthase 2 (PiCesA2) was selected as bait because of its essential role in cellulose biosynthesis and cell wall integrity[3]. Chemically synthesized AMP32 was tested for its antimicrobial activity in in vitro and ex vivo assays and showed a strong *P. infestans* growth inhibition both *in vitro* and *ex vivo*. Noteworthy, the absence of toxicity in non-target organisms was also successfully addressed. Moreover, AMP32 showed similar antimicrobial effects when tested on other oomycete species with a high homology sequence for the PiCesA2, suggesting a broad-spectrum activity. A deeper investigation has confirmed that AMP32 compromises cell viability in *P. infestans*, showing a fungistatic activity and the ability to interfere with *P. infestans* cell membrane integrity. Other undergoing studies will deepen our understanding of the structural and biochemical insights of AMP32-PiCesA interaction and mode of action (MoA) and will provide useful information for peptide modification and chemical formulation suitable for field application.

1 W. Lesseutthiphonchai, A. L. Vu, A. M. V. Ah-Fong, H. S. Judelson, How Does Phytophthora infestans Evade Control Efforts? Modern Insight into the Late Blight Disease, (2018) Phytopathology 108:8, 916-924
2 Rosa S, Tagliani A, Bertaso C, Tadini L, Visentin C, Gourlay LJ, Prizl S, Feni L, Pellegrino S, Pesaresi P and Masiero S (2023) The cyclic peptide GKCP2 enables the modulation of galactose metabolism in yeast by interfering with GAL4 transcriptional activity. Front. Mol. Biosci. 10:1017757. doi: 10.3389/fmolb.2023.1017757
3 Erwig, L., Gow, N. "Interactions of fungal pathogens with phagocytes". Nat Rev Microbiol 14, 163–176 (2016)

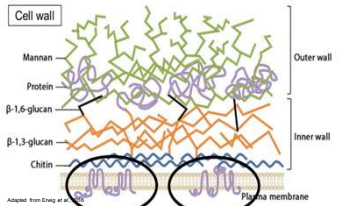
Distribution map of *Phytophthora infestans* and intensities of fungicide sprayings



Source: The CIP webpage (http://www.cipotato.org)

A

Targeting cell wall biosynthesis in Oomycetes



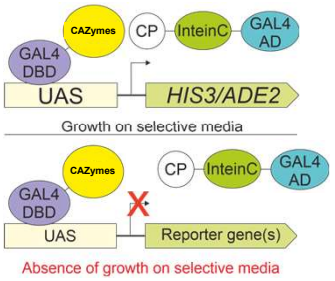
CArbohydrate en**Z**ymes (**CAZymes**):
- Cellulose synthases (CesAs)
- Chitin synthase (CHSs)
- Putative transglycosylases

WHY?

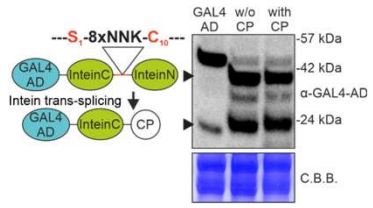
- Resistance to environmental stresses
- Mediates nutrient uptake
- Signaling interface

→ **Essential for development and infection process**

Yeast Two-Hybrid assay



Combinatorial librarY of cyCLIC peptides (CYCLIC)



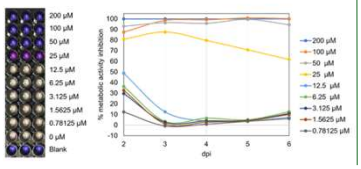
B

Bioactivity assay

Peptides identification → Peptides synthesis → Peptides testing

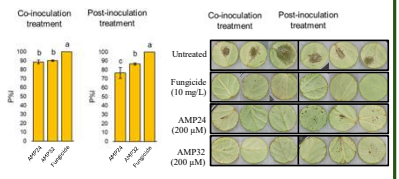
***In vitro* viability assay**

% metabolic activity inhibition of P. infestans sporangia



***Ex vivo* bioactivity assay on leaf disks**

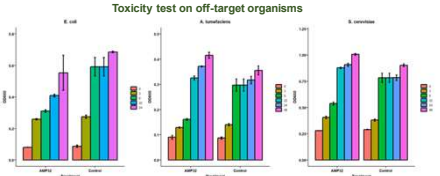
% reduction on *P. infestans*-related symptoms



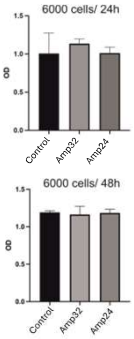
C

Toxicity test

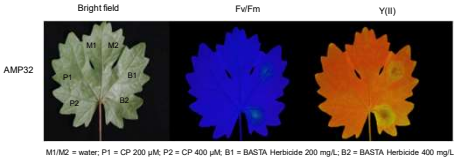
Toxicity test on off-target organisms



MTT assay on human cells



Photosynthetic performance measured using Imaging PAM on grapeine leaves

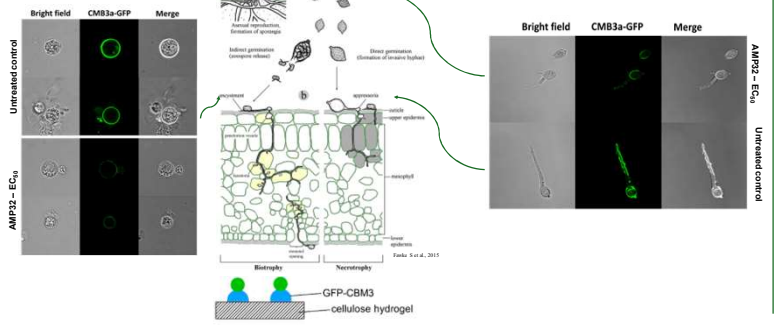


M1/M2 = water; P1 = CP 200 µM; P2 = CP 400 µM; B1 = BASTA Herbicide 200 mg/L; B2 = BASTA Herbicide 400 mg/L

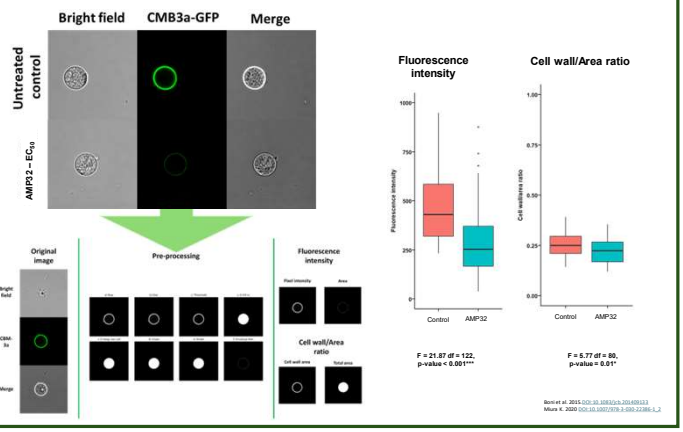
D

Molecular characterization

AMP32 interfere with PiCesA2



Cellulose quantification



Outcome: identification of peptides with antimicrobial activity for a sustainable agriculture

Bibliography:

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