Antifungal peptides to combat 'Botrytis Bunch Rot' disease in grapes

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Background

Botrytis cinerea **causes "Botrytis Bunch Rot" (Gray Mould Rot) in fresh horticultural crops, on more than 1400 cultivated plant species and causes crop yield losses $10 billion to $100 billion around the world.[1]**

In New Zealand wine industry, it is the major pathogen and costs up to NZ$5000/ha in direct crop losses and an additional NZ$1500/ha in control costs.[2]

Chemical treatments has threatened to Food safety and human health as well as contribute to emerging resistant types of pathogen, alternative safer methods have been tried. But they have not given total effect to control pathogen

Objectives

1. Synthesis of Antimicrobial peptides PAF-32 and Battacin analogues and evaluate effect of AMPs on gray mold pathogen, *Botrytis cinerea*.
2. Evaluation of the mode of action of the peptides in suppression of this fungal pathogen.
3. Evaluation of edible coating on grapes using effective peptides and their activity on the host defense and overall quality of the grape berries.

Methods and Materials

Synthesis of Antimicrobial Peptides → Purification by HPLC → Mass Spectrometry

Preliminary Bioassays → In vitro tests: Mycelial & conidial inhibition → In vivo tests: Disease Severity & Disease incidence

Ultrastructural Changes in the Fungal Mycelium due to interaction of AMPs → Scanning electron microscope (SEM) & Transmission electron microscope (TEM)

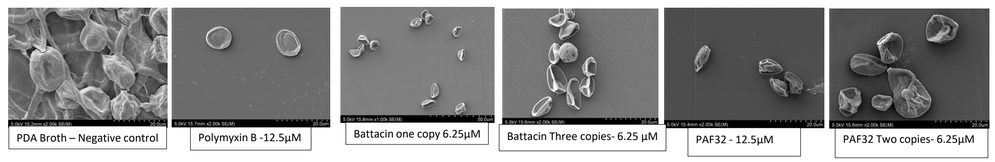
Results

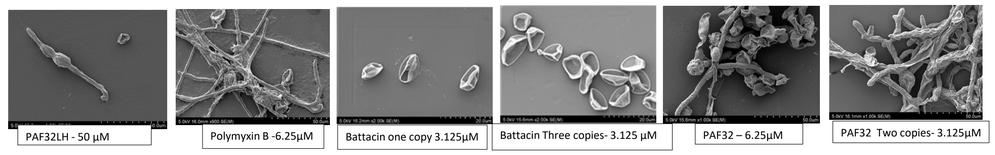
Spore germination assay (after 72 hrs)

PDA Broth (x20)	Battacin one copy - 3.125 µM (x20)	PAF32 one copy - 6.25 µM (x20)
Polymyxin B- 12.5µM (x20)	Battacin Three copy - 3.125 µM (x20)	PAF32 Two copy - 6.25 µM (x20)

Treatment	Minimal Inhibitory Concentration (MIC)
Battacin 7-Analogue - One copy peptide	3.125 µM
Battacin 7-Analogue - Three copy peptide	3.125 µM
PAF-32 -One copy	12.5 µM
PAF-32 -Two copy	6.25 µM
PAF-32 -One copy with L-Histidin peptide	50 µM
Polymyxin B –Positive control	12.5 µM
Potato Dextrose Broth (PDA)–Negaitive control	----

Scanning Electron Microscope Images





Future work

intracellular localization of AMPs in pathogen cells → Peptides activity on suppression of pathogenicity

Evaluation of effect on virulence gene expressions in pathogen → Investigation of peptides mechanisms on the host defense activity

Impact

- **No cytotoxicity due to direct target for the microbes, hence contribute to global food security.**
- **Environmental friendly and safe food for consumers.**
- **More profit due to less expenses for control pathogen, as we using very low concentrations of peptides.**

Discussion and Conclusion

Battacin peptide will bind to negatively charged Lipopolysaccharide and anionic lipid, and alter the cell membrane activity,[3] while PAF32 hexapeptide will travel inside cells and binds to RNA of the pathogen.[4] These mechanisms suppress the growth of pathogen and kill the spores and mycelium cells of *B. cinerea*.

- Both AMPs may effectively causes cell lysis of the pathogen through disrupting membrane permeability and cellular toxicity in *Botrytis* fungal mycelium and spores.
- Interactions of both AMPs with host defense and physiology may improve quality of grapes by suppressing the pathogen invasion and its growth.

References

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