Wood diseases of Kiwifruit and biocontrol strategies

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INTRODUCTION

Modern kiwifruit cultivation faces numerous idiopathic threats, known as wood diseases, such as ESCA, which significantly limit both production and quality [1, 2]. These diseases primarily damage the wood of the trunk and branches, leading to a gradual and widespread weakening of the plant [2]. ESCA, also known as "Esca disease" or "Wood decay of kiwifruit," is a complex and destructive condition caused by a consortium of fungal pathogens [2]. It shares some characteristics with the esca of grapevines and exhibits both chronic and acute symptoms (*Figure* 1) [3]. Current preventive measures rely on copper-based pesticides, which exacerbate environmental issues, phytotoxicity, and the risk of pathogen resistance. Therefore, finding sustainable control alternatives is urgent. EU restrictions on agrochemical use are encouraging research into green-based solutions to replace conventional agrochemicals and foster more sustainable agriculture.

***Figure 1.*- The symptoms of Esca on kiwifruit wood and leaves are as follows: a) white rotted areas, with spongy consistency, surrounded by dark and of hard consistency necrotic streaks; b) Interveinal chlorosis of the leaves, that often developed into severe necrotic lesions and leaf curling, culminating in premature leaf shedding.**

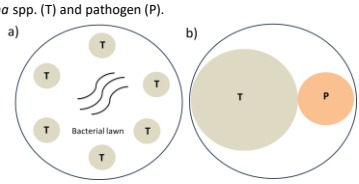
GOAL OF THE STUDY

The main goal of KiwiBol (PTDC/ASP-PLA/2440/2021) was to find new eco-friendly agents (*Trichoderma* spp. and derived peptaibols) to combat ESCA disease.

METHODS

1) Isolation of *Trichoderma* spp. from soils and rhizosphere of ESCA-symptomatic and asymptomatic orchards (North and center of Portugal) on Trichoderma Selective Medium (TSM) and taxonomic identification by sequencing of the internal transcribed spacer (ITS), RNA polymerase II gene (RPB2), and Transcriptional enhancer factor (TEF)-1 (data not shown).

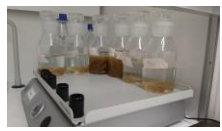
2) Evaluation of a) antibacterial activity against plant pathogenic bacteria (*Clavibacter michiganensis*, *Erwinia amylovora*, *Pectobacterium carotovorum* subsp. *carotovorum* , *Pseudomonas syringae* pv. *actinidiae*, *P. s.* pv. *syringae*, *P. s.* pv. *tabaci*, *P. s.* pv. *tomato*, *Ralstonia solanacearum*, *Xanthomonas arboricola* pv. *juglandis*, and *X. euvesicatoria*) and b) antifungal activity (fungi of ESCA complex, *Phaeoacremonium inflatipes, P. chlamydosporum, P. iranianum, P. ampelicida, Togninia minima and Bortyris cinerea*) *in vitro* using dual-culture method, *Trichoderma* spp. (T) and pathogen (P).



3) Production and quantification of crude extract, c) 15-day old cultures of *Trichoderma* were flooded with ethyl acetate and d) incubated 24 h with agitation.

c)

d)



4) Solution was then filtered, and ethyl-acetate was evaporated using a e) rotavapor to produce a dry crude extract. Next, crude extracts were analyzed by f) liquid chromatography-mass spectrometry (LC-MS).

e)

f)

RESULTS

***Table 1.* – Evaluation of the antifungal activity of *Trichoderma* spp. isolates against fungi of ESCA complex (*Phaeoacremonium inflatipes, P. chlamydosporum, P. iranianum, P. ampelicida, Togninia minima and Bortyris cinerea*.**

	P. iranianum		*P. chlamydospora*		*P. inflatipes*		*P. ampelicida*		*T. minima*		*B. cinerea*	
Characteristics	**T003**	**T033**	**T003**	**T033**	**T003**	**T033**	**T003**	**T033**	**T003**	**T033**	**T003**	**T033**
Pathogen's abnormal pigmentation	No	No	No	No	No	No	No	No	No	No	No	No
Trichoderma's abnormal pigmentation	No	No	No	No	No	No	No	No	No	No	No	No
Hyphal barrier (HB)	No	No	No	No	Yes	No	No	No	Yes	No	No	No
Trichoderma growth on pathogen	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No	Yes	Yes	Yes
Trichoderma fills plate	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Trichoderma sporulation on pathogen	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No	Yes	Yes	Yes
Trichoderma sporulation	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Inhibition halo from pathogen	No	No	No	No	No	No	No	No	Yes	No	No	No

T033 formed an inhibition halo on *Pectobacterium carotovorum* subsp. *carotovorum*, *Pseudomonas syringae* pv. *syringae*, *P. s.* pv. *tabaci*, *Xanthomonas arboricola* pv. *juglandis*, and *X. euvesicatoria* bacterial lawn, whereas T003 did not demonstrate antibacterial activity.

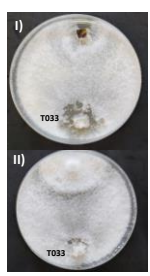


***Figure 2.* – Antagonistic potential of *Trichoderma* spp. isolate (T033) against fungi of ESCA complex (I) *T. minima* and II) *B. cinerea*.**

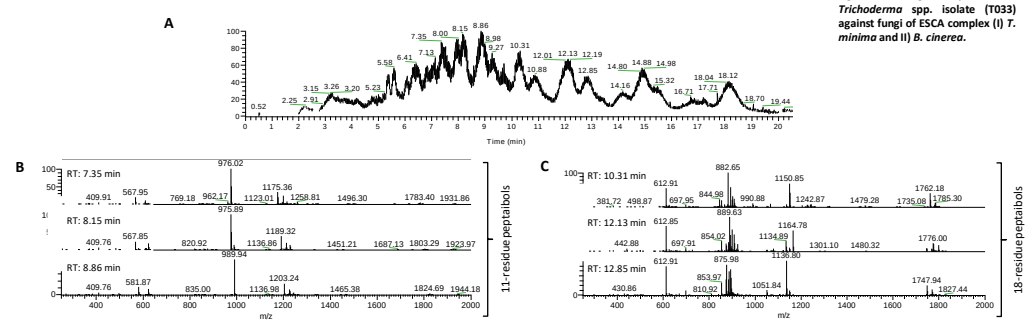


***Figure 3.* – (A) LC-MS chromatogram of T033 extract. (B) Mass spectra of 11-residue and (C) 18-residue peptaibols presented in T033 extract.**

MAIN CONCLUSIONS

- ✓ T033 demonstrated good antibacterial activity against the selected phytopathogenic bacteria.
- ✓ The most promising *Trichoderma* spp. isolates (T003 and T033), showing strong antifungal potential, were chosen for extract production and characterization to dissect the mechanism behind the antimicrobial activity;
- ✓ The analysis of T033 extract by LC-MS, revealed the presence of 11- and 18-residue peptaibols, a class of linear, amphipathic polypeptides from the fungal non-ribosomal peptide synthetase (NRPS) pathway;
- ✓ Next, we aim to synthesize bioactive peptaibols from *Trichoderma* spp. isolates, evaluate their antimicrobial activity, investigate the interactions between peptaibols and fungal membranes, and assess their biosafety. In the future this could be directly or indirectly used to control this disease.

References

[1] Beris, E., Selim, M., Kechagia, D., & Evangelou, A. (2022). Overview of the Esca complex as an increasing threat in vineyards worldwide: Climate change, control approaches and impact on grape and wine quality. In Recent Advances in Grapes and Wine Production-New Perspectives for Quality Improvement. IntechOpen.

[2] Bigot, G., Sivilotti, P., Stecchina, M., Lujan, C., Freccero, A., & Mosetti, D. (2020). Long-term effects of Trichoderma asperellum and Trichoderma gamsii on the prevention of esca in different vineyards of Northeastern Italy. Crop protection, 137, 105264.

[3] Pereira-Dias, L., Oliveira-Pinto, P. R., Fernandes, J. O., Regalado, L., Mendes, R., Teixeira, C., *et al.* (2023). Peptaibiotics: Harnessing the potential of microbial secondary metabolites for mitigation of plant pathogens. *Biotechnol. Adv.* 68, 108223. doi: 10.1016/j.biotechadv.2023.108223.

Acknowledgements

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KIWIBOL is an integrated approach that will deliver a new and eco-friendly biotechnological tool (Peptaibols and/or Trichoderma spp.) to address Kiwifruit ESCA disease's management on Portuguese kiwifruit orchards. This will make KIWIBOL an appealing approach for future translation to the field and increase the efficiency of kiwifruit production.