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Pathogen-Host Interaction Analysis of Esca-associated fungal mutant strains





J. Fischer¹. F. Fontaine³ and E. Thines^{1,2}

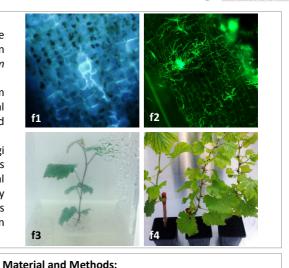
- 1 Institut für Biotechnologie und Wirkstoff-Forschung gGmbH, Erwin-Schrödinger-Straße 56, 67663 Kaiserslautern 2 Mikrobiologie und Weinforschung am Institut für Molekulare Physiologie, Johann-Joachim-Becherweg 15, Johannes-Gutenberg Universität, 55128 Mainz In association with Florence Fontaine and the University of Reims Champagne Ardenne - Faculty of Exact and Natural Sciences, Research Unit on Grapevine and Wines in Champagne – EA 4707, Institut Georges Chappaz de la vigne et du vin en Champagne

Introduction:

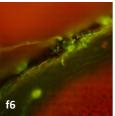
Esca is well known in the Mediterranean region and Western Europe as a destructive disease of grapevine caused by fungal pathogens showing severe symptoms on grapevine plants. Mainly Phaeomoniella chlamydospora (Pch) and Phaeoacremonium minimum (Pmi) growing as endophytes are involved (Fischer, 2015).

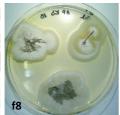
Despite the efforts made by the scientific community in the last decade, the symptom formation and the cause of those leaf symptoms is still poorly understood. In general the involvement and phytotoxic activity of the secondary metabolites scytalone and isosclerone produced by the pathogens is assumed (Andolfi et al., 2006).

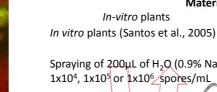
During the study the ability of several gen-deletion mutants of Esca-associated fungi (namely Pch and Pmi) to infect grapevine cuttings as well as in-vitro plants was analyzed. The previously generated mutants show a higher sensitivity to artificial stresses, such as heat or osmotic stress, than the wild type strains. Furthermore, they have a strongly reduced melanin biosynthesis because of the lack of essential enzymes (polyketide synthases, PKS) of the scytalone biosynthesis pathway. These infection assays may help to understand the host-pathogen-interaction on a molecular level.











Spraying of 200µL of H₂Q (0.9% NaC) 1x10⁴, 1x10⁵ or 1x10⁶ spores/ml

In-vitro plants

Monitoring for 7 to 14 dpi [f3]

Surface sterilisation of the plants endophyte isolation [f5, f8] microscopic evaluation [f6] using e.g. fluorescence labeled strains

Greenhouse trials Three-eye cions of Vitis vinifera cv. 'Cabernet sauvignon' (Selim et al., 2012) Spraying of 10mL of H₂O (0.9% NaCl) 1x10⁶ spores/mL [f7]

Monitoring for 28 to 90 dpi [f4]

Surface sterilisation of the plants endophyte isolation [f8] microscopic evaluation [f2] PCR evaluation of mutant stains using the HPT cassette as a marker

Results:

The results [f9] indicate that all plants tested were free of Pch and Pmi before the artificial infection assay. Furthermore, it was observed, that the WT strains and the fluorescence labeled strains had the highest infection rate in both test systems, whereas the ΔPKS strains of both fungi had the lowest reisolation rate throughout the tests. And finally, the infection rate and reisolation efforts were much lower for the greenhouse plants compared to the in vitro approach.

The impact on the infection success of the ΔPKS-mutant strains was somehow surprising since the deletion of the Tetrahydroxynaphthalene reductase (ΔT4HNR) had little to no impact on the mutants. In the future an assay that allows a monitoring of occuring symptoms due to the infection of WT and mutant strains would be a preferable research aim.

E 100 ΔT4HNR (Pm ΔPKS3 (Pml) control plan ΔPKS15 (Pch) GFP (Pmi) control plan control plant (Pch) in vitro plants

F9: results of the conducted greenhouse and in vitro plant trials

Andolfi, A., Evidente, A., Bruno, G. and Sparapano, L., 2006. Two naphthalenone pentakides from liquid cultures of «Phaeoacremonium aleophilum», a lungus associated with esca of grapevine. Phytopothologia Mediterranea, 39(1), pp.162-168. Fischer, M. and Kassemeyer, H.H., 2015. Fungl associated with Esca disease of grapevine in Germany, VITS-Journal of Grapevine Research

(AZI), p.109.

Fischer, J., Compant, S., Pierron, R.J., Gorfer, M., Jacques, A., Thines, E. and Berger, H., 2016. Differing Alterations of Two Esca Associated Fungi, Phaeoacremonium aleophilum and Phaeomoniella chlamydospora on Transcriptomic Level, to Co-Cultured Vitis vinifera L. calli. PloS

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