

Proposal for a Master Project in Microbiology

Unravelling pathogenic trade-offs in filamentous fungi exposed to a chemical stress

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Lab/Institution: Applied and Environmental Mycology lab., ITQB NOVA

Selection Method: CV and motivation letter and informal interview

BACKGROUND

Our studies have been focusing the dynamics of meta-communities of fungi, which originated from Pentachlorophenol (PCP) contaminated soils from cork oak forests. Recently, using laboratorial simulations we have identified the key PCP degraders by using amplicon sequencing upon stable isotopic probing separation (*under review*). PCP exposure altered the functioning of the metacommunity of fungi: induced major dysregulation of the carbon and nitrogen metabolisms; increased the secretion of proteins associated with pathogenesis; altered the surrounding pH, and lowered its susceptibility to fungicides. These observations suggest that PCP exposure and degradation may increase the pathogenic potential of the fungal metacommunity. The importance of these findings surpasses that of PCP exposure, since the simple structure of this persistent organic pollutant (POP) – halogenated aromatic – is found in many other life-threatening POPs and emerging pollutants.

OBJECTIVES

Our aim is to disclose the effects of acclimation to PCP and other structural related POPs, in plant pathogens.

Priority will be given to *Diplodia corticola* (Botryosphaeriaceae) - one of the most aggressive fungal pathogens of *Quercus* species; or to *Fusarium oxysporum* - pathogenic fungus with a large spectrum of plant and animals host. Knowledge on the molecular mechanisms of phytopathogenicity in *D. corticola* is still limited but recent studies have unravelled important virulence trails, and a draft of the genome annotation was made available. No data exist on *D. corticola* ability to tolerate/degrade PCP; justifying why *F. oxysporum* is herein being considered as a potential model.

Our **vision** is to understand if chemical stress may trigger phenotypic alterations that alter host-pathogen interactions, specifically triggering pathogenicity towards animal hosts.

PROJECT DESCRIPTION

The role of fungal communities in the mitigation of pollutants from soils is currently a hot-topic due to their impressive metabolic capacities. However, initial studies on the functional costs of such effort suggest an increased pathogenic potential following exposure to pollutants. The host team has been working on the topic of mitigation of pollutants for over a decade and has established a wide array of laboratorial methodologies.

The fungal strain(s) will be exposed to archetypal pollutants, namely PCP or Triclosan, along multiple generations. The **MAJOR OBJECTIVE** is to identify phenotypical and transcriptomic alterations indicative of increased pathogenicity towards animals. The student will receive continuous training and support while performing the following tasks:

Task 1) Define the dose response of the toxics against *D. corticola* and *F. oxysporum* and the degradation rate of the pollutant at 25 and 37 °C.

Task 2) Acclimation of the selected fungus to the toxics during, at least, 50 generations will be executed by consecutive sub-culturing the fungus on media containing three distinct levels of PCP (EC₅₀, EC₇₀ and EC₉₀). Two different temperatures will be used: 25 and 37 °C. Controls devoid of chemical stress will be prepared.

Task 3) Search for phenotypic alterations:

- Physiological profile (CLPPs)
- Macroscopic and microscopic transitions
- pH of the medium
- Increased resistance to other antifungals

The stability of any identified alterations will be tested by re-culturing the fungus on media not containing the toxic during 10 generations.

Task 4) Screen key transcriptomic alterations or *in vivo* susceptibility in the insect model *Galeria mellonella*. The selection will be based on the student's motivation/interest.

Task 5) Writing the dissertation.

TIMELINE

| | Month 1 | Month 2 | Month 3 | Month 4 | Month 5 | Month 6 | Month 7 | Month 8 | Month 9 | Month 10 |
|--------|---------|---------|---------|---------|---------|---------|---------|---------|---------|----------|
| Task 1 | | | | | | | | | | |
| Task 2 | | | | | | | | | | |
| Task 3 | | | | | | | | | | |
| Task 4 | | | | | | | | | | |
| Thesis | | | | | | | | | | |

Recommended literature:

Martins, C.*, Varela, A.*, Núñez, O., Martins, I., Houbraken, J.A., Martins, T.M., Leitão, M.C., McLellan, I., Vetter, W., Galceran, M.T., Silva Pereira, C., 2015. "Understanding fungal functional biodiversity during the mitigation of environmentally dispersed pentachlorophenol in cork oak forest soils". *Environmental Microbiology* 17, 2922-2934.

Martins, C.*, Varela, A.*, Silva Pereira, C., 2017. "A three-act play: pentachlorophenol threats to the cork oak forest soils mycobiome". *Current Opinion in Microbiology* 37, 142-149.

Martins, C., Varela, A., Leclercq C.C., Núñez, O., Větrovský, T., Renaut J., Baldrian, P., Silva Pereira, C., 2018. "Identifying specialisation of pathogenic trade-offs in metacommunities of fungi exposed to pentachlorophenol", *under review*

Fernandes, I., Alves, A., Correia, A., Devreese, B., Esteves, A.C., 2014. Secretome analysis identifies potential virulence factors of *Diplodia corticola*, a fungal pathogen involved in cork oak (*Quercus suber*) decline. *Fungal Biology* 118, 516-523.