

Dottorato

Sustainable Agricultural and Forestry Systems and Food Security

Title

HOST SENSING IN A CROSSKINGDOM FUNGAL PATHOGEN

Proposing tutor

David Turrà, Tenure Track Researcher (RTDB) at the Department of Agricultural Sciences, Università di Napoli "Federico II"

Objectives of the research project and interdisciplinary collaborations

Fungi belonging to the *Fusarium oxysporum* (*Fo*) species complex cause vascular wilt disease in virtually all known crop plants. Given its long-term persistence in the soil and its extensive resistance to both antifungal drugs and treatments, *Fo* is considered one of the ten most devastating plant pathogens worldwide [1-3]. I and my collaborators recently found that *Fo* uses two G-protein-coupled receptors (GPCRs) Ste2 and Ste3 to either sense self-generated fungal pheromones (α - and α -pheromone) or plant-derived cues (peroxidases), suggesting that *Fo* has co-opted and specialized its pheromone perception system to discriminate between self-pheromone peptides and host-derived signals (Figures 1) [4-7].

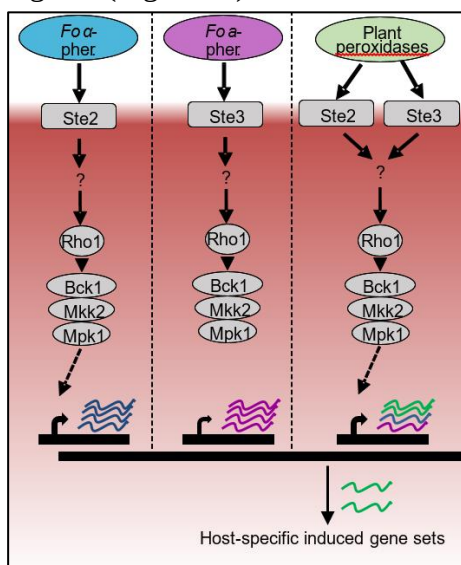


Figure 1. Flow diagram showing the research strategy used to identify host-specific genes.

We plan to perform an RNA-Seq analysis to compare the global transcriptional changes occurring in *Fo* hyphae upon exposure with self- and host-generated cues both in the wild type strain and in already available *Fo* mutants lacking either pheromone receptor (Ste2 or Ste3). This approach will identify both diverging and overlapping sets of genes controlled by these GPCRs when they either function alone (for cognate pheromone perception) or together (for host sensing) (Figure 1). Five new target genes/condition will be deleted from the *Fo* genome and their promoters fused to a fluorescent reporter gene (GFP) using protocols already established in the Antonio di Pietro (ADP) lab at the University of Cordoba (Spain). The obtained knockout mutants will be tested in both chemotropic [4, 8] and plant infection assays [9] for their ability to sense self- or plant-generated chemoattractants and to infect and colonize tomato plants, respectively. Finally, fungal reporters will be used to track via fluorescence confocal microscopy the spatiotemporal dynamics of self- and host-derived sensing in *Fo* hyphae in both chemotropic and plant infection conditions.

Innovation and originality of the project in relation to the state of the art

Parasitic *Fusarium* spp. have evolved specialized mechanisms to locate and enter the available portals present on the surface of their plant and human hosts. The molecular events occurring early during parasite-host recognition have been largely ignored in previous studies. The finding that *Fo* has co-opted a class of pheromone receptors to chemotropically sense host-derived chemoattractants, provides a solid basis for this proposal, aimed at identifying the unexplored potential of GPCRs as primary fungal regulators involved in host-sensing. The generated results will represent both a general advancement in fungal cell signaling research and a universal resource platform for efficient treatment of fungal diseases that profoundly impact both agricultural crop production and human health worldwide. Indeed, while the importance of the Ste2 and Ste3 GPCRs in pheromone-dependent regulation of cell-directionality and gene transcription has been studied in detail in the model yeast *Saccharomyces cerevisiae*, their relevance in fungal sensing and regulation of directional growth responses towards gradients of chemical compounds produced by the prey has remained largely unexplored. The identification of genes specifically activated by these GPCR during host perception will provide new insights on how pathogenic fungi transduce and reprogram their transcriptional landscape to host-derived cues, which will represent a framework for the development of yet unexplored therapeutic strategies against fungal disease.

Grant availability

Laboratory costs will be funded through the following national and regional grants from the Italian Ministry of Education, University and Research and the Campania Region (POR FESR CAMPANIA 2014/2020- O.S. 1.1 [grant number Bioagro 559]; MIUR Prin [grant number PROSPECT 2017JLN833]).

Collaborations with foreign institutions

Part of the described work, mainly the generation of fungal knock-out and fluorescent reporter mutants, will be conducted by the selected Ph.D. student in collaboration with the Antonio di Pietro's (ADP) lab at the University of Cordoba (UCO; Cordoba, Spain), a pioneer in genetic transformation and molecular dissection of signaling in *Fo*. The participating institutions (UNINA and UCO) will bring in complementary competencies which will ensure the smooth fulfilment of the proposed tasks.

References (in bold publications authored by the tutor)

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8. Turra, D., et al., ***Hyphal chemotropism in fungal pathogenicity***. *Semin Cell Dev Biol*, 2016. **57**: p. 69-75.
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