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Catching cereal killers: a multi-omics approach to disentangle yeast-Fusarium interactions in the phyllosphere

Gouka, L.

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Summary

The overarching goal of this PhD thesis is to investigate the taxonomic and functional diversity of yeasts in the wheat phyllosphere and their interactions with the mycotoxigenic fungal pathogen *Fusarium graminearum*. This work combines *in vitro*, *in vivo*, and *in planta* bioassays with genomic, transcriptomic, and metabolomic approaches to explore the functional potential of phyllosphere yeasts for disease control and food safety.

The phyllosphere, the aboveground part of a plant, is a complex microbial habitat characterized by exposure to both biotic stresses, such as pathogen invasion, and abiotic stresses, such as nutrient limitation, temperature fluctuations and exposure to ultraviolet light. Despite these harsh conditions, yeasts successfully colonize the phyllosphere and possess specific adaptive traits to thrive in this challenging environment. These traits include the ability to utilize a wide range of carbon sources, form biofilms, and withstand environmental stresses such as desiccation and high temperatures. Through extensive genomic analyses, this thesis highlights the functional diversity of yeasts in the wheat phyllosphere, with particular emphasis on the metabolic pathways that enable stress tolerance and pathogen antagonism. Comparative genomics identified key traits in phyllosphere yeasts, including genes involved in the synthesis of VOCs, and enzymes that degrade complex polysaccharides. These findings highlight the underexplored functional potential of environmental yeasts associated with plants.

Fusarium graminearum is a devastating pathogen of wheat, causing Fusarium head blight (FHB) and producing mycotoxins such as deoxynivalenol (DON) that contaminate cereal crops, posing health risks to humans and livestock. The use of biocontrol agents, such as yeasts, is an increasingly important strategy for managing FHB and reducing mycotoxin contamination, while minimizing fungicide use. The results of this thesis demonstrate that several phyllosphere yeast species exhibit strong antagonistic activity against *F. graminearum*, both through direct growth inhibition and via the production of VOCs. VOCs produced by the yeast species *Aureobasidium pullulans* and *Metchnikowia* spp. inhibited fungal growth and reduced FHB disease severity. The putative VOCs involved in these activities include 2-phenylethanol, phenylacetaldehyde, dimethyl disulfide, and dimethyl trisulfide. These findings highlight the significant role of VOCs-mediated interactions in the antagonistic activity of the selected yeast species.

To better understand the molecular mechanisms underlying yeast-pathogen interactions we uncovered how specific yeast species modulate pathogen growth and mycotoxin production. Analysis of the transcriptional responses of both yeasts and *F. graminearum* to VOC exposure revealed that certain VOCs impact the biosynthesis of mycotoxins in *F. graminearum*, either by degrading toxin precursors or inhibiting their biosynthesis. This yeasts' ability to produce antimicrobial VOCs and to decrease mycotoxin accumulation in wheat grains opens new avenues for developing sustainable agricultural practices that reduce reliance on chemical fungicides and mycotoxin-decontamination treatments. However, several challenges remain, particularly in understanding the full diversity and ecological roles of yeasts in the phyllosphere, the molecular mechanisms behind their antagonistic properties, and the practical application of yeast-based control strategies in the field. Future studies are needed to explore the spatiotemporal distribution and survival

of yeast species in the phyllosphere, identify their stress-adaptive traits, and characterize the many unknown biosynthetic gene clusters we discovered in the novel genomes of the environmental yeasts. Technological advances in genomics, transcriptomics, and metabolomics will be critical for uncovering the full functional potential of yeasts in plant protection, with the ultimate goal of integrating these microorganisms into agricultural practices for enhanced crop resilience and sustainable food production.