Competition in the Phyllosphere: Epiphytic Yeast Dispersal and Community Assembly in Tomato Phyllosphere

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* Presenting

Abstract

Fungi living within and on leaf surfaces play an integral role in plant functional processes including resistance to pathogens. The microbial interactions occurring at these interfaces often mediate pathogen protection or facilitation within the plant host. Foliar yeasts are a particular group of unicellular fungi that have been extensively studied for their antagonistic nature towards foliar and fruit pathogens. Less studied, however, is the way yeasts associate with other microbes and persist in the phyllosphere. This knowledge is integral to assessing their effectiveness and viability as biocontrol agents towards plant pathogens over diverse biotic and abiotic conditions. In this study we characterize various competitive traits of foliar yeasts isolated from tomato, *Solanum lycopersicum*, leaves, including dispersal and in vitro antagonism (dual cultural assays). To visualize the species interactions of three dominant epiphytic yeast in the tomato phyllosphere (*Sporobolomyces roseus, Filobasidium magnum*, and *Sporobolomyces nylandii*), we performed an in planta epiphytic mycobiome inoculation and through temporal sampling tracked the assembly, dispersal, and competition of the yeast.

Introduction

In a previous microbiome passaging experiment, *S. roseus* and *S. nylandii* were found to have a negative density dependence in the phyllosphere (Morella et. al 2020). A wild type microbiome was passaged over four generations of tomato. Initially, *S. roseus* was the dominant fungal species, but by the end of passage 4, *S. nylandii* had become the dominant species with *S. roseus* at undetectable levels. This inverse abundance relationship became the source of investigation, with the hypothesis that *in planta* these yeast species may be competitors.

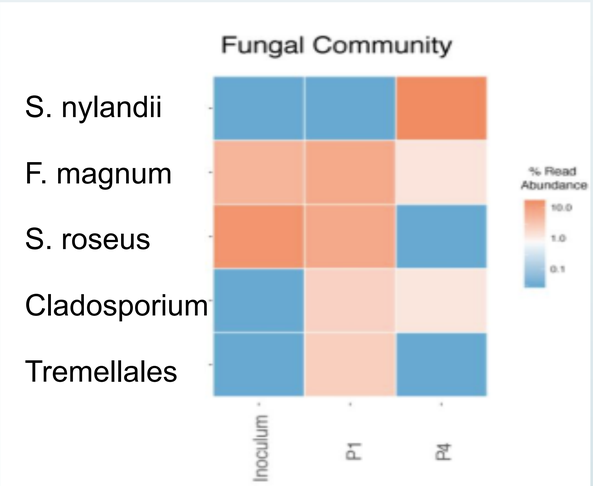

Figure 1: Relative abundance of fungal community in wild type inoculum, at the end of passage 1 (P1) and end of passage 4 (P4). (Morella et. al 2020).

In vitro Direct Inhibition Assay

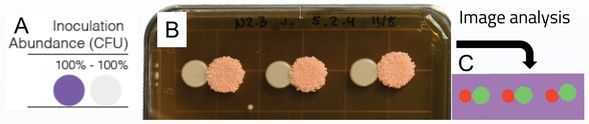

Figure 2: A) schematic of plating for direct inhibition assay. B) F. magnum (left) plated against S. nylandii (right) no direct inhibition observed. C) ImageJ was utilized to quantify radial growth rate of fungal strains and data was used to produce Heatmap below.

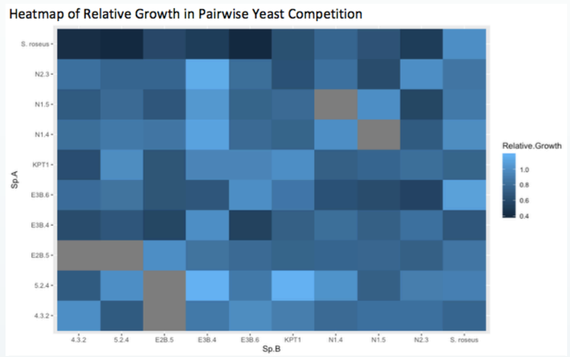

Figure 3: Left to right Sp. A relative growth when competed against Sp. B, diagonal is the control. Gray indicates lack of data.

No direct inhibition was observed in vitro across any of the strains tested. *S. roseus* is a poor competitor in nutrient rich, petri dish environment, due in part to its slow growth rate.

In planta Density-dependent inoculation and Sampling Methods

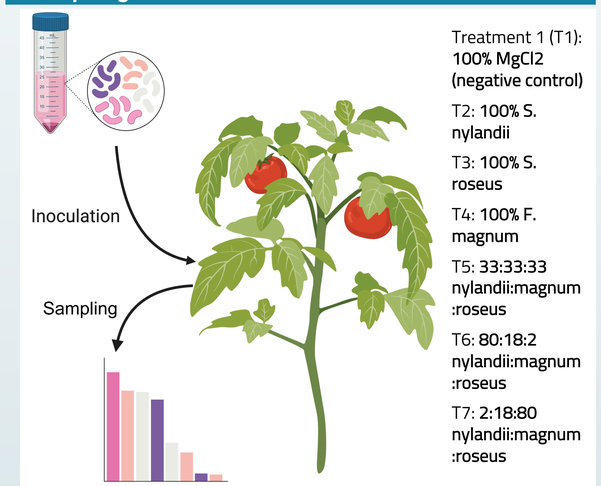

Figure 4: In planta density-dependent mycobiome inoculation methods schematic. 7 treatments of varying densities of three fungal species were inoculated on tomato plants with a total of 4 replicates per treatment. Plants were placed in a humdifying chamber for 24 hours after inoculum spray to ensure microbiome establishment on the leaves. An abbreviated sampling regime was conducted due to campus closure from COVID-19. Day 0 (before inoculum spray), Day 1 (24 hours after inoculum spray), and Day 7. Three leaves were harvested at each sampling timepoint and the microbiome was extracted by sonicating the leaves in a leaf-wash buffer. Leaf wash was plated and allowed to grow for 4 days before CFU counts were taken, distinguishing the fungal species by their morphology.

In planta Results

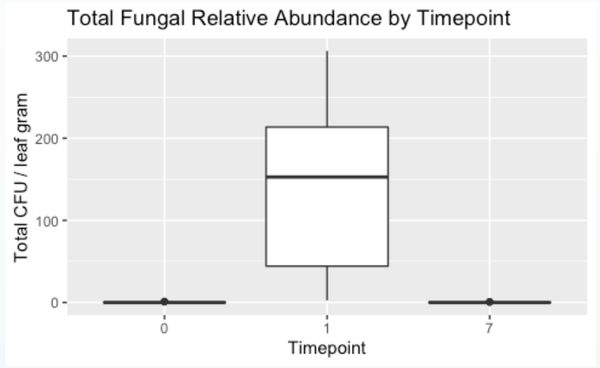

Figure 5: Total CFU's across time indicating relative fungal community abundance at each sampling timepoint. CFUs were standardized across plants by weight of the sampled leaves.

The given sampling method was not suited to quantify the established yeast community. Day 1 high density of yeast identified was more representative of remaining inoculum existing in the droplets on the leaf surfaces and dispersal occurring within the misting chamber, than actual established fungi in the phyllosphere (days 0 & 7).

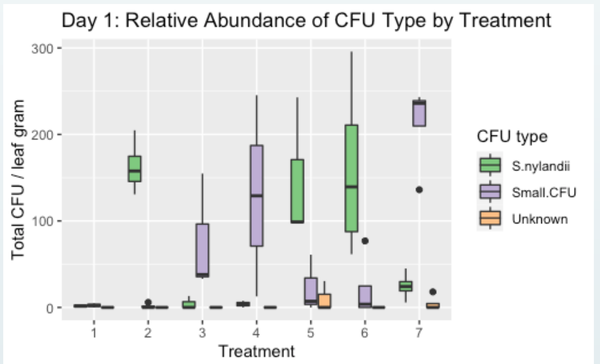

Figure 6: Relative Abundance of CFU type by treatment. CFU types were categorized by yeast morphology. Small CFU represents S. roseus and F. magnum which were indistinguishable at the time of CFU counts.

Due to campus closure from COVID-19, CFU counts were performed on dilution series plates where 20 uL of leafwash was plated and allowed to grow for up to 4 days. There was not sufficient time for yeast to grow and differentiate S. roseus from F. magnum, thus they are described together as "Small CFU."

S. nylandii was found in all samples, irrespective of treatment type.

Conclusion

Among the characteristics important for fungal establishment and persistence in the phyllosphere are direct inhibition of competitor species, dispersal, and growth strategies. By pairwise direct inhibition assays, there was no observed *in vitro* antagonism. This along with with the relatively gradual fluctuation of abundances in the fungal community in the passaging experiment indicate that toxin secretion is not a strategy among the species tested for fungal dominance in the phyllosphere. There were drastic differences in growth rate of the fungal strains *in vitro*. Most notably, S. roseus was a slow grower, which may account for its lower competitive ability *in vitro*. The high nutrient and environmental stability of the petri dish is a different environment than the harsh, nutrient poor, and highly variable environment of the phyllosphere. In order to test whether the pattern of S. nylandii outcompeting yeast *in vitro* is conserved in the natural environment, a density-dependent mycobiome inoculum experiment was performed. The significant differences between total yeast abundance in days 0 and 7 compared to day 1 may be due to the sampling method that was not resolve enough to capture the established yeast in the phyllosphere. Fungal abundances at day 1 represent excess inoculum as well as dispersal that occurred across plants within the humidity chamber. While all three species tested were ballistospore forming yeasts, a unique group of yeast that are capable of dispersing their cells by ejecting them from the parent fungus, only S. nylandii was found across treatment types in day 1, indicating that its enhanced dispersal in the humidity chamber may play an important role in its dominance of the phyllosphere over time.

The extenuating circumstances of campus closure from COVID-19 interrupted the *in planta* experiment; however, concentrated leaf wash from all timepoints have been preserved and may be revived for more accurate CFU counts and further experimentation at a later time.

Whether the differences in relative abundance observed in the passaging experiment of Morella et. al (2020) were due to these yeast species occupying the same niche and competing in the phyllosphere or changes in the phyllosphere environment across the seasons remain open questions and are opportunities for further investigation.

Acknowledgements

1) Koskella lab
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Morella, N, Weng, F, Joubert, P, Metcalf, J, Lindow, S, & Koskella, B 2020, "Successive passaging of a plant-associated microbiome reveals robust habitat and host genotype-dependent selection", *PNAS*, Jan 2020, 117 (2) 1148-1159.