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T3: Adaptive assembly of cotton root microbiome in gel microdroplet-based microfluidic platform

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Abstract

Our understanding of the ecological adaptation mechanisms that result in root microbiome (rhizobiome) composition, diversity and structure is elementary. Our aim was to improve foundational knowledge of how rhizobiome changes dynamically over time in response to abiotic and biotic perturbations and how we may be able to lead this adaptive assembly in our favor. In this study, we focused on investigating cotton rhizobiome components surrounding *Fusarium oxysporum vasinfectum* race 4 (Fov4). This highly virulent pathogen was recently introduced into Texas, and the risk of the disease spreading to other fields is a major concern. We isolated bacteria from cotton rhizosphere and tested anti-Fov4 activities. Our study provided evidence that bacterial volatile organic compounds (VOCs) can stimulate or suppress physiological behaviors in Fov4 and other co-inhabiting bacterial isolates. Notably, different combinations of VOCs exhibited different impact on Fov4 growth. Fov4 inoculum density was influenced by cotton varieties in the field, with the role of abiotic factors on inoculum density yet to be determined. Lastly, we designed a microfluidic chamber array chip for high-throughput phenotype testing, which can allow us to test the impact of multiple abiotic and biotic factors on Fov4 physiology.

Introduction

Recent technological advances have uncovered tremendous organismal diversity in crop-associated microbiomes, especially in the root microbiomes (also known as rhizobiomes). While these advances have also resulted in detailed characterization of rhizobiomes, our understanding of the ecological adaptation mechanisms that result in rhizobiome composition and structure is rudimentary. In addition, how functional holobiont (the functional entity formed by a macrobe and its associated microbes) adapts in response to abiotic and biotic perturbations remain unknown. To study these mechanisms, we targeted the rhizobiome in a currently unfolding plant disease crisis due to the recent emergence of F. oxysporum f. sp. vasinfectum race 4 (Fov4). This highly virulent fungal strain on Pima cotton was recently introduced into Texas, and the possibility of the disease spreading to Upland cotton production fields is a major concern.

Our project goal was to elucidate the dynamic mechanisms employed by cotton rhizobiome holobionts to adapt to biotic and abiotic perturbations. To analyze and model how biotic and abiotic factors impact the cotton rhizobiome structure adaptive assembly, we first sampled multiple soil samples, and isolated bacteria exhibiting anti-Fov4 activity. We investigated the mode of communication between Fov4 and bacterial isolates. We studied cotton research field for Fov4 inoculum density in different cotton varieties. We also developed and tested a microfluidic system that can rapidly test a multitude of different experimental combinations.

Objective 1: Establishing a synthetic cotton rhizosphere

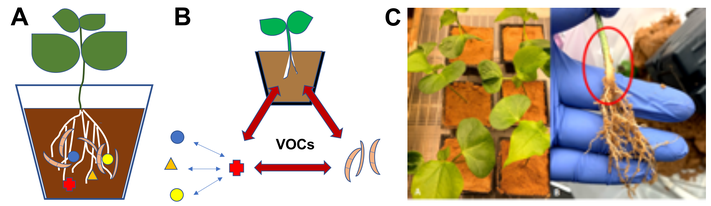

Fig. 1. (A) Schematic depiction of a synthetic cotton rhizosphere with Fov4 (canoe-shaped spores) and 4 bacterial isolates. **(B)** Overview of our hypothetical assembly of cotton, Fov4, and bacteria, where VOCs are identified as potential communication tool. **(C)** Cotton growth stage with base of shoots where bacterial were harvested.

Table 1. Bacterial isolates exhibiting anti-Fov4 activity. Soil samples from Pima cotton fields in El Paso county as well as non-cotton growing Harris county were screened.

Isolate ID	Predicted species	% Identity
ELP529	*Paenibacillus ehmensis*	99
ELP528	*Paenibacillus tianuensis*	96
ELP524	*Paenibacillus elgii*	97
ELP745	*Streptomyces schreyahn*	95
RZ141	*Bacillus amyloliquefaciens*	93
Rz160	*Bacillus subtilis*	99
HC658	*Streptomyces fradiae*	95

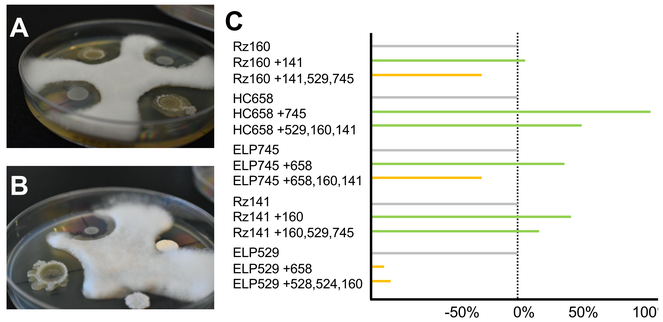

Fig. 2. (A&B) Multiple bacterial isolates on agar plates with FOV4 inoculated to test anti-fungal activity. **(C)** Relative comparison of inhibition zone when additional isolates were inoculated on plates, suggesting that bacteria are using VOCs to stimulate or suppress physiological behaviors on other isolates

Objective 2: Abiotic factors impacting disease epidemiology

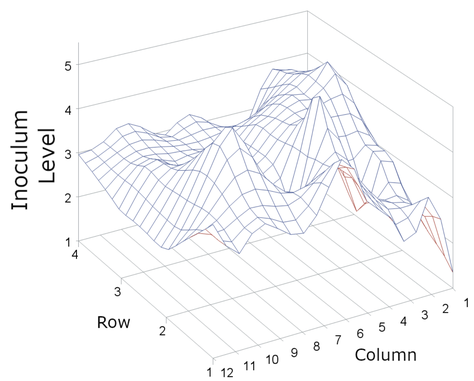

Fig. 3. Inoculum density variation in a cotton research field in El Paso. The result showed that there is a significant variation in inoculum density in the field. Analysis further showed that different varieties affect inoculum differently. We are now observing environmental variation that may affect inoculum density variation.

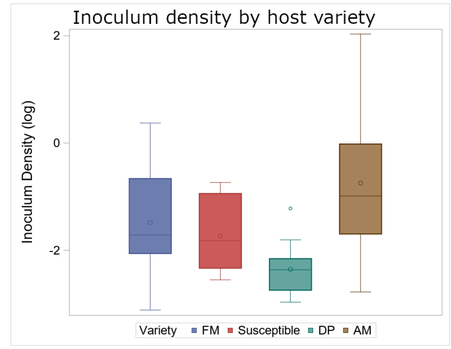

Fig. 4. Box plot for relative inoculum density variation from the same cotton research field. The data highlights four varieties to show that they are associated with different inoculum levels at the end of an experiment. Some varieties amplify inoculum, and others are associated with decrease in inoculum.

Objective 3: Microbiome modification testing with high-throughput microfluidics

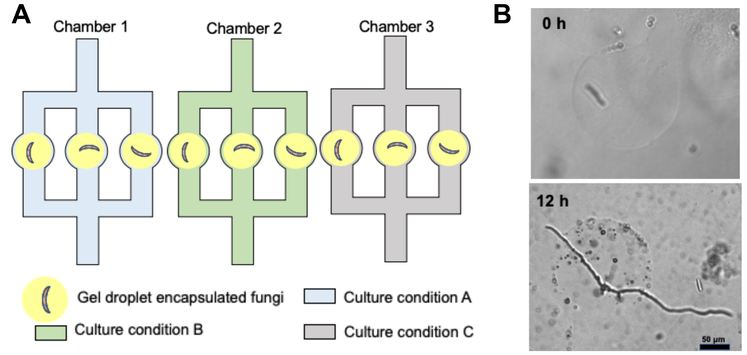

Fig. 5. (A) A microfluidic chamber array chip. We designed a microfluidic chamber array chip which contains 3 chamber array, each array can accommodate single agarose gel droplet containing single fungi. This array allows simultaneous testing 3 different culture conditions at single cell resolution with three replicates. This array can be easily expanded to contain hundreds and thousands of chambers to perform high-throughput phenotype screening. **(B)** Growth of encapsulated fungi. We tested the growth of encapsulated single filamentous fungi spore in YEPD. During culture, fungi reached out of gel and grow into filamentous shape.

Summary

The outcomes from this project will help improve our foundational knowledge of the networked interactions in microbiomes, including how they change dynamically over time and in response to biotic and abiotic perturbations. For instance, our finding that bacterial VOCs serves as an important communication tool in Fov4 rhizobiome, for fungal-bacterial and bacterial-bacterial associations, that can help elucidate the fundamental mechanisms/strategies that lead to the adaptive assembly of a tolerant rhizobiome.

The knowledge and data generated from this study allowed PIs to submit multiple expansive USDA NIFA grant proposals in 2020. PIs thank T3: Texas A&M Triads for Transformation funding for the opportunity to initiate this collaborative research.