

A New Synthetic Community System for Studying Microbial Interactions Driven by Exometabolites

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<http://ashley17061.wixsite.com/shadelab/synthetic-microbial-communities>

<http://ashley17061.wixsite.com/shadelab/plant-associated-microbiota>

Project Goals: 1) Determine how interactions among microbial community members are underpinned by chemical interactions within their community. 2) Understand the consequences of and feedbacks on member gene regulation for microbial community interactions by linking changes in member transcripts to community exometabolite production.

Microorganisms exist in communities of species that can interact chemically, and these chemical interactions underlie a range of relationships from commensalism to antagonism¹⁻⁴. Yet there is little known about how microorganisms interact with each other in their native habitats, or how these microbe-microbe interactions scale up to impact community outcomes. Because of the specificity of many known microbe-microbe relationships, it is thought that most microorganisms produce certain chemical products only within a particular community^{1,5,6}. It follows that investigating a microorganism in isolation offers a narrow perspective of the full spectrum of its metabolic potential. Therefore, we use a simple and advantageous synthetic microbial community system to interrogate chemical interactions among microorganisms (via exometabolites, signaling molecules and other extracellular compounds), allowing us to observe behaviors that only occur when those microorganisms exist as part of a particular consortium.

In the synthetic community system, microbial members are arrayed randomly into a 96-transwell plate with 0.22- μ m filter-bottoms (per well) that physically separate each member from its neighbors but permits resource and metabolite exchange through a shared media reservoir. Community exometabolites from the media reservoir are extracted with a protocol that captures a variety of molecules, which are then analyzed using sensitive mass spectrometry. We use the system to determine how community exometabolite composition and dynamics change given particular member combinations and/or experimental treatments. In addition, we quantify member outcomes using live-dead staining with flow cytometry.

We conducted two experiments to demonstrate the synthetic community system's efficacy⁷. The first experiment showed the system's potential for capturing antagonistic

interactions via an antibiotic, and the second experiment showed the system's potential for capturing synergistic interactions via a shared signaling molecule. Next, we demonstrated the system's potential by assessing community exometabolite changes in a three-member community over time in stationary phase⁷. For this demonstration, community members included typical environmental strains with relevance for plant-soil-microbiome interactions: *Pseudomonas syringae* DC3000, *Chromobacterium violaceum* ATCC 31532, and *Burkholderia thailandensis* E264. We observed directional changes in community exometabolite production over stationary phase, supported by highly reproducible biological and technical replication. We also linked the production of some exometabolites to certain members or member combinations. For example, *B. thailandensis* was associated with many of the most dynamic and consistent mass spectral features. We found evidence for a previously undescribed antagonistic member interaction, as there consistently were reduced live cell counts of *P. syringae* when grown in the same synthetic community as *B. thailandensis*. Finally, our results suggest that microbial interactions facilitated by exometabolites do not necessarily have an outcome for members' population sizes, suggesting that nuanced microbial interactions may be overlooked if population changes are the only data considered.

Ultimately, this project seeks to integrate -omics approaches to understand dynamics of microbial interactions within their communities, with an overarching goal of understanding how these interactions translate to community function. In doing so, we will understand how specific member combinations determine collective community outcomes. We have established this synthetic community system as a reproducible laboratory model that will provide a tool to uncover interactions among members of engineered or environmental microbial communities. Future directions include collaboration with the Joint Genome Institute through a Community Science Project to link member gene expression to community exometabolite production.

References

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