Will Sharpless
July 2020

SPUR Proposal: Environmental Control of Microbiomes

Research Statement

Microbiome states have been correlated with health and disease across biological kingdoms: unique organizations of microbes can influence obesity, brain diseases and behavior in humans [1, 2, 3] and pathogen resistance, drought resilience and bountiful yield in agriculture [4]. I propose to interrogate how the interactions of a library of agricultural microorganisms will change in regard to resource scarcity to illustrate how the state and stability of the root microbiome might fluctuate in these contexts. This would be a subproject within Professor Adam Arkin's mission to design an optimal microbiome for rice plant growth, and would demonstrate which organisms are best suited in a variety of contexts. I will use a droplet-based culture method to quantify the pairwise interactions of our 150 members and will publicize the interaction parameters in an online database afterward for other microbiome researchers. Additionally, I will incorporate dynamic interactions within the theoretical control framework proposed by the Liu group [5] to illustrate how to minimally control a microbiome. In general, the work would shed light on the behavior of agricultural microbiomes and how to provide the best environment to control them.

Background and Rationale

With the acceleration of microbial community research, the field has produced accurate models and methods for designing communities to achieve the aforementioned functions [6, 7, 8], but the models are parameterized on microbial interactions which are treated as constant values. A major criticisms of microbiome research is that the test tube environment does not properly mimic the natural, and, therefore, the research does not commute to application. However, I hypothesize that the discordance originates in the interactions and growth rates because they are functions of setting, and I will thus see large variation in the community topology and steady state as the environment varies. I propose to quantify and model the microbiome in response to real environmental stresses, which farmers see on their plot as they exhaust resources or apply copious fertilizer, to expect how the research transfers to the field.

It is commonly understood that microbes change their behavior in response to a lack of carbon by making antibiotics and other secondary metabolites [9, 10]. If we model the microbiome with the generalized Lotka-Volterra model [6, 7, 8], we understand the alteration in behavior, for example, a tendency toward aggression, can cause the stability of the community to shift. This can lead to dominance of pathogenic members as well as collapse of the entire community. However, the aggression could be beneficial for destroying a pathogen: when induced, *Pseudomonads* will make toxins that benefit the plant by killing fungal pathogens like *Rhizoctonia solani* [11], the cause of sheath leaf blight, a plant disease that is estimated to cost the US $22 million/year [12]. In general, frameworks for controlling the microbiome depend on the connections (aggressive or amiable) between the members [5], and to quantify how the network changes in different environments, as my proposal suggests, will elucidate the best context for altering a community as well as provide a method for accessing uncontrollable structures.

In the study of controlling networks, it is imperative to identify nodes, or clusters of nodes, which form autonomous elements and remain recalcitrant unless directly altered [5]. The number of autonomous elements in a microbial network implies the minimum number of driver species, species which are killed or boosted to control the network, that must be affected to have full range of the microbiome state space [5]. Hence, a more connected network or a microbiome with more interactions requires fewer driver species in general. Therefore, if we can identify an environment which maximizes the network connections, we can potentially further minimize the number of driver species needed to control the microbiome.

Project Plan & Methods

I proposed the research to Adam Arkin in the spring as an independent project and had begun developing the droplet and ribosomal quantification methods with the supervision of postdoctoral researchers in the Arkin lab. We have already isolated and classified rhizosphere bacteria over the past two years and have selected 150 of these which span the diversity of our isolate library and appear in metagenomic data that our collaborators haver garnered from healthy rice. In the winter, I aided in the design and production og the droplet chamber mold with a supervising scientist in which the droplets will be combined. I completed a trial run in January and troubleshot the method in the following weeks, identifying the best method for the experiment. The COVID pandemic interrupted the process but after validating new trial runs in the next couple weeks, I will then verify the quantity of cells in each droplet and validate that the DNA barcodes in each droplet are reported correctly. Therefore, I expect to complete the preparation (Phase 0) by the end of August 2020.

The resource variation experiment (Phase 1) will include 5 concentrations of medium and if time allows, 5 concentrations of root exudate. In February, I quantified the growth rates of a sample of the library in order to find that the concentration of 3.1% Tryptic Soy Broth (TSB) resulted in growth rates similar to those reported in situ, and the range will span 2 log units. After completing this set of experiments and sequencing analysis (Phase 1 and Phase 2) before September, I will assay the interaction variance in root exudate collected in the fall from mature rice plants in 5 concentrations, in order to see if certain interactions and growth are selected by the plant.

For parameterizing the model (quantifying the interaction under a certain condition), I will follow former lab member, Assistant Professor Ophelia Venturelli's method which resulted in prediction of 12 member communities with $r^2 = 0.91$ [6]. The method requires sampling the interactions over 50 hours and parameter fitting by bayesian regression and L1 regularization of the timeseries data. With 150 isolates and their 11,175 total pairwise interactions, and a droplet generator which can make 2,000,000 droplets in one pool, I can take 7 time points and have ~25 replicates of each time point for parameter fitting. The droplets then undergo PCR and can be sequenced in parallel. I will use Dr. Song's Python program for associating the DNA barcodes and getting ribosomal counts. During the months of COVID, I have written my own parameterization code for my experiment based on the structure of Dr. Venturelli's scripts and validated it on her data sets. Therefore, I expect that the droplet experiments (Phase 1) and modeling (Phase 2) will take 2 months and will conclude in early October.

We expect the droplet method has some bias; therefore, I will be completing a method identical to Dr. Venturelli's for pairwise analysis of 12 members in 200 ul cultures in 96 well plates. While this is more tedious than the droplet method, it is paramount to quantify the biases for publication in a peer-review journal. I have practiced with a Biomek fluidic handling robot in the lab in order to streamline experimentation and increase throughput, and I will use Dr. Venturelli's Biomek programs for pairwise coculture. I have selected 12 of the 150 that represent the rice rhizosphere and I will begin pairwise parameterization in the upcoming weeks with the expectation of finishing in September.

I will have October and November to interpolate the interactions as functions of resource concentration, and then validate that communities of the organisms follow the model predicted dynamics at the corresponding TSB concentrations (Phase 3). Similar to pairwise coculture, this will entail 200 microliter cultures with approximately 3 communities in triplicate. Finally, I will use the parameter functions to demonstrate that the quantity of minimal drive species varies with resource concentration. The work should be accomplishable, but the possibility of difficulties is nonzero. I have outlined several goals, some of which are to be expected and others possible if all else succeeds without delay. The ultimate goal of the project is to publish the work in a peer-reviewed Journal before my graduation in December 2020.

In regard to the COVID19 pandemic, the research will be conducted with the utmost caution by abiding to the regulations set in place by the university and LBNL and is fortunately well suited for social distancing and remote work. The research is particularly suited for the pandemic because of several factors: the majority of the hands on wet lab work has already been completed (isolates chosen, confirmed and stored), the droplet experiments are fairly simple in preparation (they only require a long time on a special lab-owned machine), the validation experiment will be carried out by a robot, thereby minimizing human-to-human exposure, and the modeling can be done from my desk at home. Additionally, the machines, the droplet generator and the Biomek robot are kept at an Arkin lab space that is partitioned from other laboratories at Potter street, a less populated and open lab area. Therefore, the work will easily abide by safety regulations of the university and will remain so even if the restrictions become more stringent.

Qualifications and Project Affiliations

I am a double major in Applied Mathematics and Microbial Biology and my relevant coursework includes classes on ME 132 Dynamical Systems and Feedback, EE 222 Nonlinear Systems, E150 Modeling for Industrial Research and PMB 112 General Microbiology. I have worked in the Arkin lab for 2 years now, and previously worked in Jay Keasling's lab for 18 months where my work resulted in four publications. While I would be working under Post Doctoral researchers Kyle Sander and Fangchao Song, I have pursued independent projects before: a colleague and I won 3rd place in the UC Berkeley Big Ideas Competition for our proposal and attempt to engineer polyethylene degrading bacteria.

The proposed work falls under the Center for Utilization of Biological Engineering in Space (NASA) collaboration between UC Berkeley, Stanford, Utah State, UC Davis and University of Florida (NASA Grant Award Number NNX17AJ31G). The design of optimal rice communities is specifically a mission goal (which my project aligns with) and collaboration within CUBES

between the Coleman-Derr lab (UCB) and the Arkin lab, and we maintain steady communication with them, sharing research materials and metagenomic data.

Budget

The budget is outlined in the following figure. The project is fortunately limited to the expansion of experiments the lab plans to undergo regardless of my project, both the plate validation and droplet experiments, to various media. Therefore, the base supplies required for generating droplets, doing genome extractions, polymerized chain reactions etc. are already established, rather, the costs below originate from the various media types I will use along with extra plates, Biomek supplies and qPCR reagents which I will use extensively.

Item Description	Product Number	Number	Price per Unit	Total Cost
$(NH_4)_2SO_4$ (for culture media)	A4418-500G	1	$71.60	$71.60
$MgSO_4 \cdot 7H_2O$ (for culture media)	230391-500G	1	$41.30	$41.30
$FeSO_4 \cdot 7H_2O$ (for culture media)	215422-250G	1	$51.80	$51.80
$MnSO_4$ (for culture media)	M7634-100G	1	$59.30	$59.30
K_2HPO_4 (for culture media)	P3786-100G	1	$48.30	$48.30
Biomek® 2000 P250 Tips, with Barrier, 960 case	BK140505	1	$324.77	$324.77
Half Reservoir, 24 case	BK372786	1	$145.09	$145.09
Quantitative RT-PCR ReadyMix™, 200 rxns	QR0200-1KT	1	$417.00	$417
100 pack of 96-Well plates, clear, sterile	CLS9018BC-100EA	1	$520.00	$520.00
tax				7.00%
shipping				10.00%
			Project Total:	$1,976.37

References

1. Rosenbaum, Michael, et al. “The Gut Microbiota in Human Energy Homeostasis and Obesity.” Trends in Endocrinology & Metabolism, vol. 26, no. 9, 2015, pp. 493–501., doi:10.1016/j.tem.2015.07.002.

2. Lyte M. (2014) Microbial Endocrinology and the Microbiota-Gut-Brain Axis. In: Lyte M., Cryan J. (eds) Microbial Endocrinology: The Microbiota-Gut-Brain Axis in Health and Disease. Advances in Experimental Medicine and Biology, vol 817. Springer, New York, NY

3. Johnson, Katerina V.-A., and Kevin R. Foster. “Why Does the Microbiome Affect Behaviour?” Nature Reviews Microbiology, vol. 16, no. 10, 2018, pp. 647–655., doi:10.1038/s41579-018-0014-3.

4. Lakshmanan V., Ray P., Craven K.D. (2017) Toward a Resilient, Functional Microbiome: Drought Tolerance-Alleviating Microbes for Sustainable Agriculture. In: Sunkar R. (eds) Plant Stress Tolerance. Methods in Molecular Biology, vol 1631. Humana Press, New York, NY

5. Angulo, M.T., Moog, C.H. & Liu, Y. A theoretical framework for controlling complex microbial communities. Nat Commun 10, 1045 (2019). https://doi.org/10.1038/s41467-019-08890-y

6. Venturelli, Ophelia S, et al. “Deciphering Microbial Interactions in Synthetic Human Gut Microbiome Communities.” Molecular Systems Biology, vol. 14, no. 6, 2018, doi:10.15252/msb.20178157.

7. Coyte, K. Z., et al. “The Ecology of the Microbiome: Networks, Competition, and Stability.” Science, vol. 350, no. 6261, May 2015, pp. 663–666., doi:10.1126/science.aad2602.

8. Kehe, Jared, et al. “Massively Parallel Screening of Synthetic Microbial Communities.” Proceedings of the National Academy of Sciences, vol. 116, no. 26, Nov. 2019, pp. 12804–12809., doi:10.1073/pnas.1900102116.

9. Ghoul M., and Mitri S.. 2016. The ecology and evolution of microbial competition. Trends Microbiol. 24:833–845.

10. Hibbing M. E., Fuqua C., Parsek M. R., and Peterson S. B.. 2010. Bacterial competition: surviving and thriving in the microbial jungle. Nat. Rev. Microbiol. 8:15–25

11. Nandakumar, R., Babu, S., Radjacommare, R., Raguchander, T., & Samiyappan, R. (2002). Pseudomonas fluorescens mediated antifungal activity against Rhizoctonia solani causing sheath blight in rice. *Phytopathologia Mediterranea, 41*(2), 109-119.

12. Tsiboe F., Nalley L. L., Durand A., Thoma G., Shew A., 2017. "The Economic and Environmental Benefits of Sheath Blight Resistance in Rice," Journal of Agricultural and Resource Economics, Western Agricultural Economics Association, vol. 42(2).

13. Revsbech, N., Pedersen, O., Reichardt, W. *et al*. Microsensor analysis of oxygen and pH in the rice rhizosphere under field and laboratory conditions. *Biol Fertil Soils* 29, 379–385 (1999). https://doi-org.libproxy.berkeley.edu/10.1007/s003740050568