In situ sequencing for deciphering spatial taxonomic structures of plant-associated microbial communities.

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Project Goals:
We aim to develop methods for characterizing microbial communities with single-cell and spatial resolution. Here, we develop a fluorescent *in situ* sequencing (FISSEQ) technology[1-3] for acquiring the spatial arrangement of bacteria to better understand how plant microbiomes impact host physiology.

Most investigations reduce microbial physiology to monoculture conditions, which does not consider their abundant interactions in natural environments. Indeed, much of our understanding of microbes stems from gene deletions, heterologous expression, and *in vitro* enzyme characterization. Microbes need to be studied *in situ* where their spatial organization holds biological importance for responding to their environment. However, *in situ* characterizations of these complex phenotypes in natural environments are generally not feasible at scale and remain largely unknown.

Here, we describe our recent developments for the taxonomic identification of microbes with single-cell and spatial resolution. We have established *in situ* hybridization (ISH)-based probes that can distinguish between sequences with as little as one nucleotide difference and with unique barcodes (i.e., unique molecular identifier [UMI]) for highly-multiplexed *in situ* sequencing readouts. For method development, we improve the nucleotide discrimination of our probes to multiple positions that can be discerned by a high-fidelity ligase, expanding the number taxa that we can assay simultaneously. We then demonstrate our technology by assaying 25 diverse microbes and demonstrate spatial single-cell resolution with Sequencing by Combinatorial Hybridization. Notably, our library generation and Expansion Microscopy methodology is applicable to a wide range of bacteria with different cell walls and cell morphologies. We can differentiate several members of the same genus (e.g., within *Pseudomonas*, *Bacillus*, *Lactobacillus*, *Staphylococcus*, and *Borrelia* genera), demonstrating that our technology can distinguish between bacteria that standard FISH methods cannot.

We are now applying this technology to investigate the spatial distribution of a 185-member synthetic community on Arabidopsis roots in collaboration with the Dangl Lab[4]. While our technology was developed for spatial taxonomics, we are also pursuing an additional simultaneous sequencing modality; spatial transcriptomics of the Arabidopsis host to investigate the change in spatial gene expression in response to microbial community structure. To this end, we have demonstrated Expansion Microscopy of Arabidopsis root tips, assembled full-length ribosomal DNA genes from draft genome assemblies using pathracer, and are working towards assaying bacterial localization using a universal probe and gene expression of control mRNA targets.

This work forms the framework for investigating the taxonomic structure of microbial communities and is the foundation for future *de novo in situ* sequencing of microbes in natural environments.

References

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