

Deploying ProBac-seq to dissect the flagellin regulons in plant-associated *Sphingomonas* at the single-cell level

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Abstract:

Plants monitor their environment for microbial invaders using pattern-recognition receptors that detect microbe-associated molecular patterns (MAMPs). Flagellin, the primary component of the bacterial flagellum, contains the flg22 epitope recognized by the plant immune receptor FLS2. Immune recognition creates an evolutionary conflict, requiring bacteria to balance flagellar function with immune evasion. Previous studies demonstrated that plant-associated *Sphingomonas* resolve this constraint by partitioning motility and colonization functions between two divergent, independently acquired flagellin genes. Comparative genomics revealed the widespread coexistence of two divergent flagellin genes encoding either an immunogenic variant (FliC-H) or a nonimmunogenic variant (FliC-L). The nonimmunogenic FliC-L is necessary and sufficient for full directional swimming, whereas FliC-H is dispensable for swimming but sufficient for full attachment and colonization. Flagellin expression patterns are consistent with these functional distinctions. These findings raise several important questions about how this specialization is encoded at the gene-expression level: whether individual bacteria express FliC-H or FliC-L exclusively or simultaneously, whether auxiliary flagellum genes are co-expressed with their cognate FliC, and which additional genes define the regulon underlying each variant's function. To address these questions, we are deploying probe-based bacterial single-cell RNA sequencing (ProBac-seq), a method that pairs large oligonucleotide probe sets with 10x Genomics droplet microfluidics. Within this platform, we developed an 8-sample multiplexing scheme that substantially reduces the overall cost of profiling multiple conditions in a single run. This approach will define the regulons underlying FliC-H and FliC-L expression, revealing the broader transcriptional programs that accompany each flagellar strategy and how they are coordinated with the colonization-versus-motility trade-off that governs FLS2 surveillance.