Genetic Systems to Enable Biosystems Design in Rhizosphere Pseudomonas

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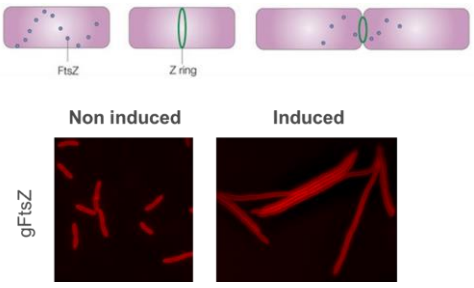
Project Goals: The goal of the Environmental Sensing and Response SFA is to understand the molecular mechanisms associated with plant-microbe interactions, particularly those interactions that lead to Plant Growth Promotion (PGP). With a focus on deciphering the PGP mechanisms mediated by rhizobacteria, we are developing/adapting genome editing tools based on recombineering and CRISPR-Cas9 systems for the *Pseudomonas fluorescens* group. Our aim is to accelerate Biosystems design by streamlining the construction of multiple mutations in any *P. fluorescens* strain.

Highly sophisticated and powerful genetic approaches exist in Pseudomonads that are based on transposon mutagenesis and conjugational transfer from *E. coli.* However the design and construction of targeted mutations with these approaches require numerous steps that are hardly amenable to high-throughput. In a context where our aim is to interrogate the mechanisms of plant growth promotion in rhizobacteria by systematically testing predictions generated through data integration and modeling and to manipulate these mechanisms to modulate PGP effects, genome editing tools providing a simple and consistent way to design and construct mutations across *Pseudomonas* strains are necessary.

Recombination-mediated genetic engineering (Recombineering) relies on the homologous replacement of host genome sequences by an incoming DNA fragment. Recombineering is efficiently promoted by bacteriophage-encoded recombination functions which accommodate short homologies. It is highly amenable to high-throughput as demonstrated by the development in *E. coli* of Multiplex Automated Genome Engineering (MAGE) by the Church lab.
We have developed a recombineering system based on a bacteriophage recombinase from *P. fluorescens* SBW25 genome. This system was used to construct knockouts of transporters in SBW25 (see P. Korajczyk poster) and to integrate unique combinations of fluorescent proteins and antibiotic resistance markers in four environmental strains of *P. fluorescens*.

In the CRISPR-Cas9 system, the sequence specificity of the nuclease is programmed by a small RNA. CRISPR-Cas9 has been applied for bacterial genome engineering where it provides a strong selection to recover cells carrying the desired mutations. We are using a catalytically inactive variant of CRISPR-Cas9 (dCas9) which binds its DNA target and prevents transcription, thus providing a tool to control gene expression. This CRISPRi system was used to downregulate various *Pseudomonas* genes involved in cell division and signaling (see Figure). Future work with CRISPRi will involve systematic down-regulation of genes and pathways for validation of functional hypotheses and model predictions.



CRISPRi-mediated depletion of FtsZ in Pseudomonas. Induction of dCas9 in cells results in the depletion of an essential cell division protein and leads to dramatic cell filamentation.