Genetic Control of Plant Root Colonization by the Biocontrol agent, *Pseudomonas fluorescens*

Benjamin J. Cole[1], Meghan Fletcher[2], Jordon Waters[3], Kelly Wetmore[3], Matthew J. Blow[1], Adam M. Deutschbauer[3], Jeffry L. Dangl[2] and Axel Visel[1]

[1]DOE Joint Genome Institute, Walnut Creek, CA 94598
[2]University of North Carolina, Chapel Hill, Chapel Hill, NC
[3]Energy Biosciences Institute, Berkeley CA

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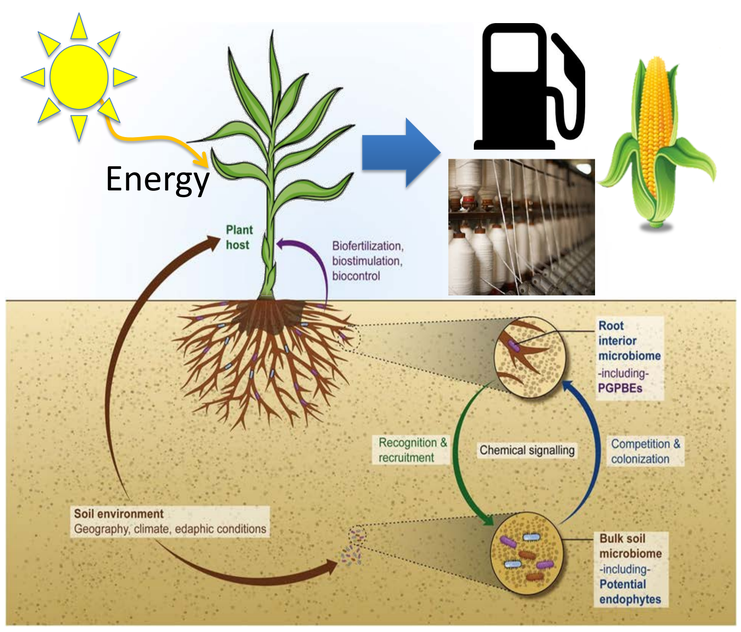
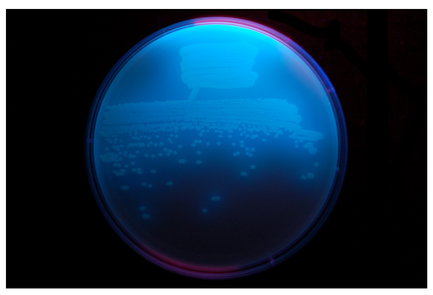
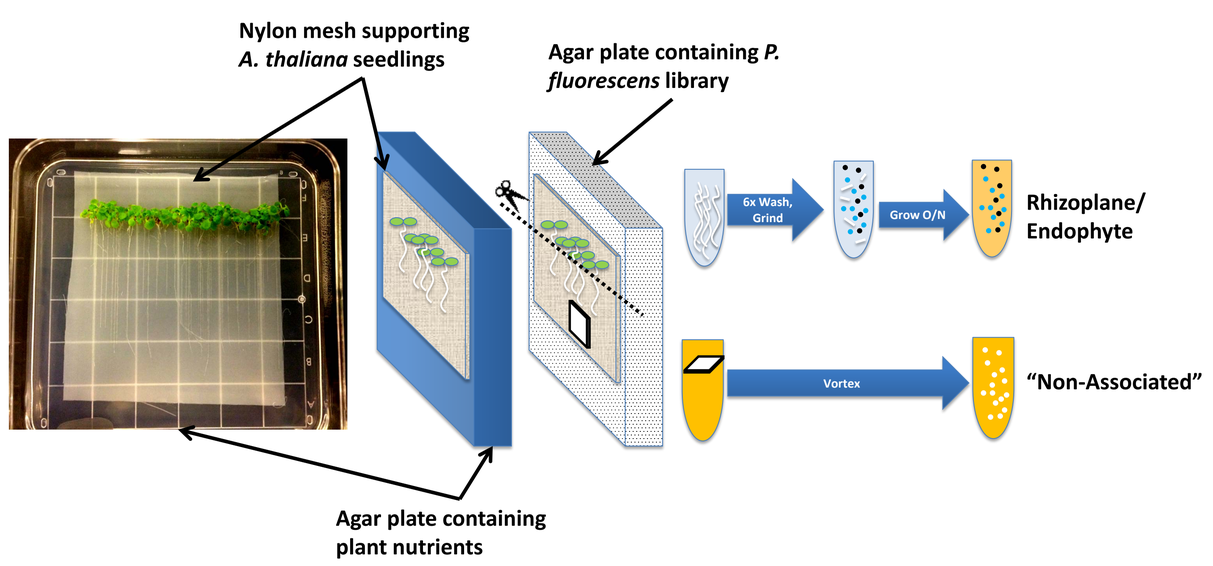
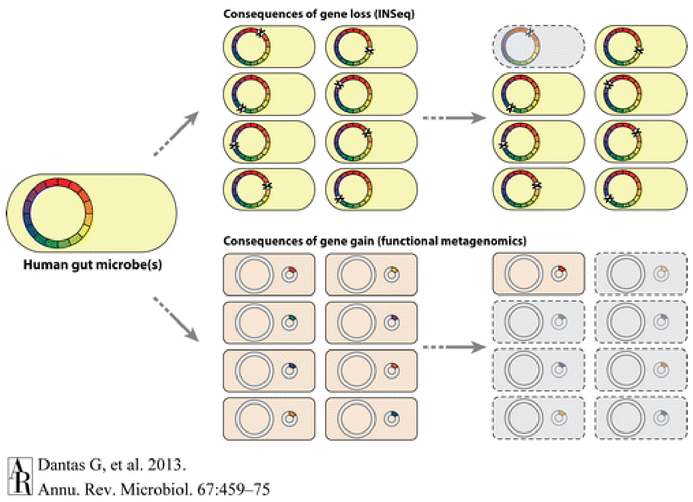
Plant growth promoting rhizobacteria (PGPR) are a critical component of plant root ecosystems. PGPR promote plant growth by solubilizing inaccessible minerals, suppressing pathogenic microorganisms in the soil, and directly stimulating growth through hormone synthesis. *Pseudomonas fluorescens* is a well-established PGPR isolated from wheat roots that can also colonize the root system of the model plant, *Arabidopsis thaliana*. We have created barcoded transposon insertion mutant libraries suitable for genome-wide transposon-mediated mutagenesis followed by sequencing (TnSeq). These libraries consist of over 10^5 independent insertions, collectively providing loss-of-function mutants for nearly all genes in the *P.fluorescens* genome. Each insertion mutant can be unambiguously identified by a randomized 20 nucleotide sequence (barcode) engineered into the transposon sequence. We used these libraries in a gnotobiotic assay to examine the colonization ability of *P.fluorescens* on *A.thaliana* roots. Taking advantage of the ability to distinguish individual colonization events using barcode sequences, we assessed the timing and microbial concentration dependence of colonization of the rhizoplane niche. These data provide direct insight into the dynamics of plant root colonization in an *in vivo* system and define baseline parameters for the systematic identification of the bacterial genes and molecular pathways using TnSeq assays. Having determined parameters that facilitate potential colonization of roots by thousands of independent insertion mutants in a single assay, we are currently establishing a genome-wide functional map of genes required for root colonization in *P.fluorescens.* Importantly, the approach developed and optimized here for *P.fluorescens>A.thaliana* colonization will be applicable to a wide range of plant-microbe interactions, including biofuel feedstock plants and microbes known or hypothesized to impact on biofuel-relevant traits including biomass productivity and pathogen resistance.

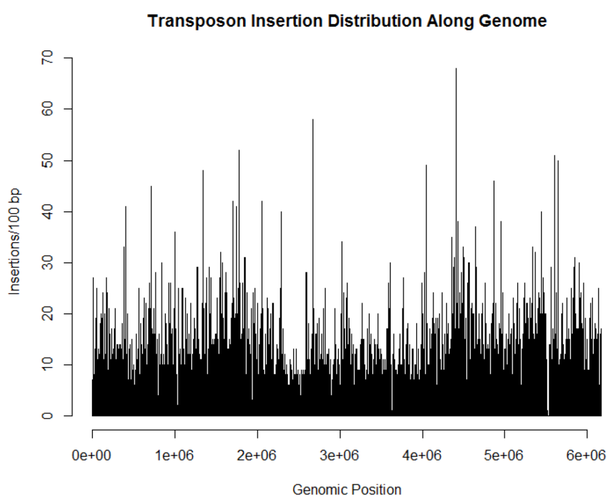






Plant growth-promoting rhizobacteria occupy various niches within the soil. Ultimately understanding their function will improve plant yields, benefiting bioenergy crops. *A. thaliana* (top right) is a well-studied model plant. Ease of transformation, a sequenced genome, simple genetics and growth requirements make *A. thaliana* (left) a superb model plant species for studying complex soil interactions. *P. fluorescens* (bottom right) is a well-studied plant growth-promoting microbe, originally isolated from wheat as an anti-fungal agent.



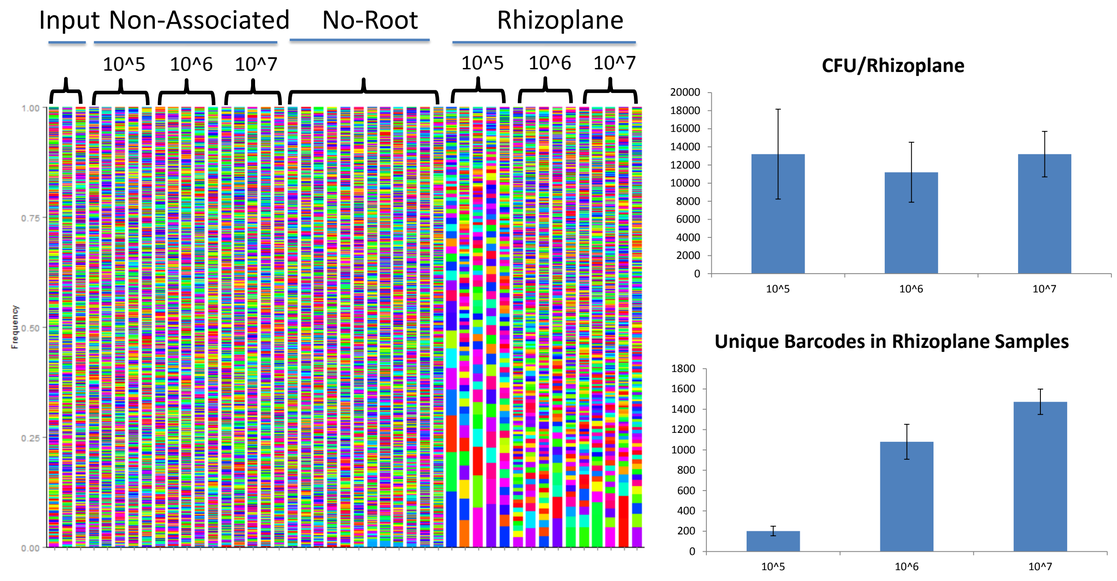


P. fluorescens TnSeq library:

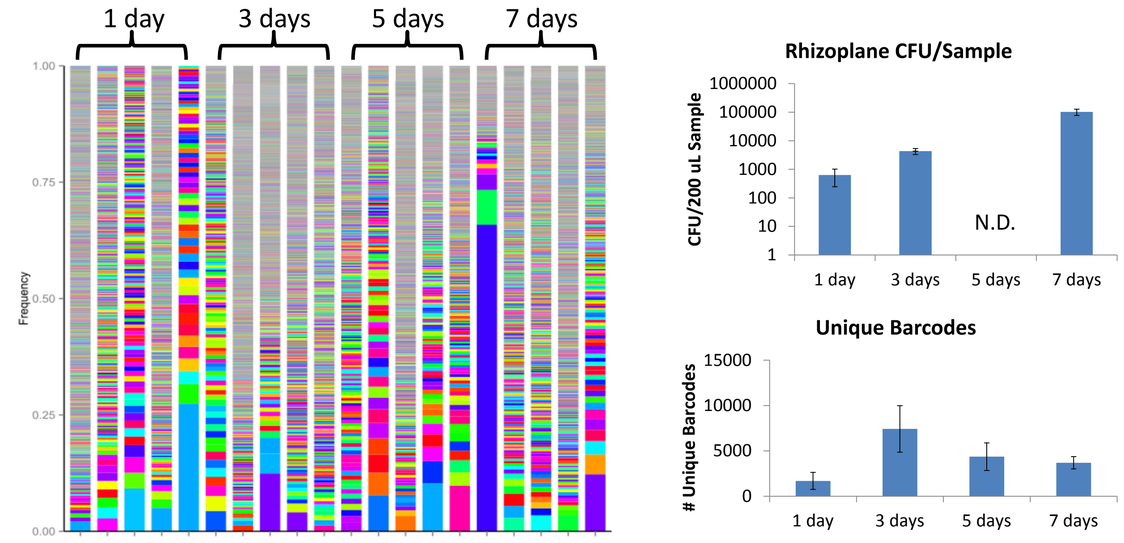
- 110,142 insertions
- 5,069 genes disrupted
- ~15 insertions/gene

Tnseq is an emerging method for functional genome annotation. Here, an insertion mutant library is challenged with a given condition, and insertion mutants that survived are identified. Genes underrepresented in the final population are candidates for facilitating growth in this condition. We constructed an insertion library containing over 10^5 randomly barcoded transposons spanning the *P. fluorescens* genome

Concentration Dependence of colonization complexity



Time Dependence of colonization complexity



7 day old *A. thaliana* roots were co-cultivated with the bacterial library. Infected roots are then isolated, washed, and cultured. For comparison, a piece of the agar substrate containing non-colonizing bacteria is also isolated and cultured. When the barcodes linking the transposon to the insertion site were sequenced, a strong concentration (starting bacterial titer) dependence was observed, while the time of co-cultivation had little effect.

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