

223. Exploiting Plant Growth-Promoting Root Endophytes to Improve Feedstock Nitrogen Acquisition

Meghan E. Feltcher¹, Natalie Breakfield², Hunter Cameron¹, Sur Herrera Paredes¹, Surge Biswas¹, Amanda Panousis¹, and Jeffery L. Dangl¹

¹University of North Carolina, Chapel Hill

http://labs.bio.unc.edu/dangl/projects/Functional_endophyte_manip.htm

Project Goals: It is increasingly clear that sustainability of plant feedstocks will require novel approaches to maintaining plant fitness in diverse soil environments with fluctuating nutrient and moisture levels. Plant health is co-dependent on the microbial communities intimately associated with roots (the root microbiome), which can positively modulate plant physiology by increasing bioavailable nutrients, stimulating root growth through phytohormone signaling, and priming the plant innate immune system to enhance pathogen resistance. A major goal of the Dangl lab is to define members of the root microbiome that are functionally relevant to plant health and productivity. Furthermore, we want to determine how these beneficial bacteria compete amongst a complex soil microbial community to occupy the plant root niche and confer plant growth-promoting activity. Because nitrogen is the limiting element of net primary production in most terrestrial ecosystems, we are interested in determining how root-associated bacteria can stimulate plant growth under limited nitrogen conditions. More specifically we want to 1) identify endophytic bacteria that stimulate plant growth under nitrogen stress and define the mechanisms by which plant biomass is increased, 2) determine the bacterial traits required for root endophytic compartment colonization and whether occupying this niche increases the success of plant-growth promoting bacteria in community settings, and finally, 3) define synthetic bacterial communities that confer robust plant resilience to nitrogen stress.

Plants employ several strategies to maximize nitrogen uptake amidst fluctuating soil nitrogen levels, such as root architecture modulations, regulation of nitrogen assimilation pathways, and maintenance of a root microbiota that improves root nitrogen acquisition. Exploiting this root microbiome in order to improve plant fitness is an attractive strategy for generating fertile crops while reducing dependence on chemical fertilizers. Specifically, there has been interest in isolating plant growth-promoting bacteria (PGPB) that improve plant nitrogen acquisition. Unfortunately, efforts to inoculate field crops with PGPB often fail, likely due to competition with native soil microbial communities and limited root endophytic compartment (EC) colonization efficiency. Thus, there is a demand to study EC colonization by PGPB both in the context of the root microbiome community and changing nitrogen conditions. Our group, in collaboration with the Department of Energy Joint Genome Institute (DOE-JGI), completed a bacterial census of the EC community of *A. thaliana* grown in two geographically distinct soils using 16S ribotyping. An important finding from this study was that the microbial composition of the root EC was both taxonomically distinct from and less diverse than that of bulk soil, suggesting there are plant- and/or bacterial- derived factors governing root EC community assemblage. To begin to understand what members of the root microbiome (specifically, endophytes) contribute to plant fitness under nutrient stresses and pathogen challenges, we isolated a diverse collection of putative endophytes from *A. thaliana* roots grown in the same two natural soils. This collection of nearly 600 isolates provides the basis for studying the root microbiome in a more reductionist laboratory setting, allowing us to develop screens for various plant growth-promoting activities and to construct synthetic soil and root communities that can be manipulated and studied under various nutrient stresses.

We hypothesize that some of these putative EC colonizers stimulate plant growth under nitrogen stress;

therefore, we are screening this diverse collection of putative root endophytes in *A. thaliana* mono-association experiments for both EC colonization and plant growth-promotion activity. Using a gnotobiotic vertical growth method on agar plates with varying nitrogen concentrations, we are able to monitor both bacterial-induced changes to rosette size as well as root architecture. Screening of over 60 isolates revealed that bacteria from diverse families stimulate rosette growth under sufficient nitrogen conditions such as Microbacteriaceae, Nocardiaceae, Burkholderiaceae, and Bacillaceae. However, many of these growth-promoting strains resulted in reduced *A. thaliana* shoot biomass under nitrogen stress. Those that increased rosette size under nitrogen deprivation include isolates from the Pseudomonadaceae and Comamonadaceae families. These mono-association studies have also revealed that many isolates robustly colonize the *A. thaliana* EC. To understand the bacterial genes required for EC colonization of diverse PGPB identified in these screens, we are using transposon mutagenesis coupled with high-throughput sequencing (TnSeq). Additionally, we are comparing PGPB to other taxonomically-related isolates in our collection (defined by 16S) in order to identify phenotypic diversity that could be exploited in whole genome sequence comparisons to look for the genetic determinants of growth promotion.

We have also constructed diverse synthetic communities of up to 53 isolates to determine how individual plant- growth promoting bacteria perform amongst a community, and to begin to identify root community structures that maximize plant growth. We demonstrated that re-colonization of *A. thaliana* roots is robust in our model community system and some synthetic communities can promote *A. thaliana* growth. Another important finding is that some isolates that colonize the EC robustly in mono-association experiments do not associate with the root in synthetic community experiments. This suggests they are outcompeted by other members of the community. Interestingly, several isolates that stunted *A. thaliana* growth in mono-association were unable to do so in the context of a community. This result suggests that other isolates either directly dampen bacterial activities detrimental to plant growth or stimulate the plant in a protective way. Current work is aimed at using mono- association data to construct synthetic communities in order to determine if individual PGPB activities are additive and transferable to more complex model systems (such as plants grown in pots with synthetic or natural soils). Also, to elucidate the mechanisms by which PGPB are stimulating plant biomass under nitrogen deprivation we are measuring root architectural changes associated with increased rosette size, and will use RNAseq analysis to determine how the plant nitrogen stress response is changed upon addition of PGPB. Results from these studies will further our understanding of the dynamics of natural root microbiome structures that directly impact the health of field crops, therefore aiding in the development of novel PGPB-based strategies that can compete in natural microbial soil communities to increase crop performance in an environmental friendly matter.

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