Plant-Microbe Interfaces: developing a synthetic community system to test preferential allocation to nitrogen-fixing bacteria

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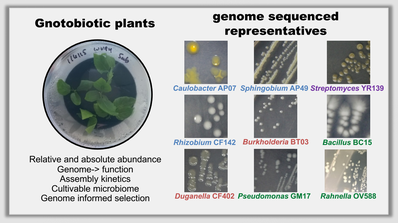
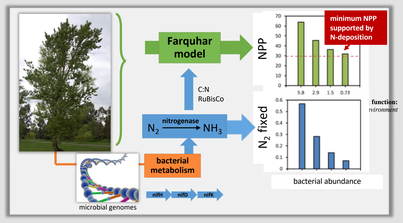
Abstract

Plant-microbiome function results from complex interactions among microbial members, host plant genetics/physiology and surrounding environmental conditions. Once the plant – microbiome is established (after initial colonization events), a key question is whether the host plant can actively discriminate among mutualists by 'rewarding' beneficial members through preferential allocation of carbon. To begin to address this question, we developed and characterized reference microbiomes of 10 bacterial strains representing abundant and functionally diverse orders identified, isolated and genome-sequenced from natural *Populus* microbiota including potential diazotrophic strains. Subsequently we investigated the ability of five diazotrophs to colonize and function in a *P. trichocarpa* host. After three weeks of co-culture conditions, our results showed strain-specific preferences for plant organs and tissues as indicated by CFUs and qPCR analyses. *Rahnella* sp. OV588 was determined to be a robust colonizer of *Populus* tissues and a *nifH* deletion mutant was generated for that strain for further functional characterization. Using a germ-free magenta box system with calcined clay substrate, *P. trichocarpa* genotype 819 was either uninoculated or inoculated with wild-type OV588 or a *nifH* deletion mutant. All experimental combinations were provided with either Hoagland's complete nutrient medium (with N) and without N. In the no N condition, plants cultured with OV588 showed a 48% increase in total plant dry weight relative to uninoculated plants or plants with the *nifH* mutant Strain. Furthermore, acetylene reduction assays of whole *Populus* plants showed ~5-fold increase in ethylene production when colonized by wild-type OV588 compared to uninoculated or *nifH* mutant-inoculated plants. There were no significant differences in ethylene production, total dry weight or chlorophyll concentration when N was included in the growth medium regardless of the bacterial inoculum. Experiments using $^{15}N_2$ gas in the plant growth chamber are underway. Our work here suggests that N_2 is being fixed by *Rahnella* sp. OV588, which contributes to enhanced plant growth under N-limiting conditions. Future studies will use a dual label of $^{13}CO_2$ and $^{15}N_2$ within a split root system to address questions of preferential allocation within a community context.

Linking phytobiome genetics to system function

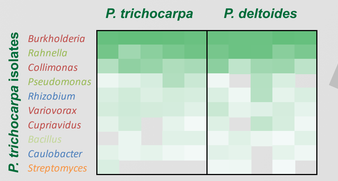
Controlled laboratory studies to predict ecosystem function



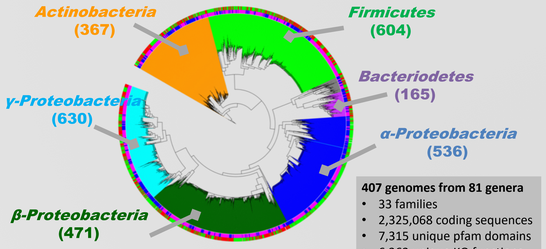


Left: axenic plants are generated through tissue culture protocols developed at ORNL. **Center:** Bacterial isolates collected from native *Populus* trees. **Right**: Mesocosms for sterile plant-microbe culture

PMI reference communities



Are communities…
…consistent across replicates?
…consistent across host species?
…dictated by phylogeny?



407 genomes from 81 genera
- 33 families
- 2,325,068 coding sequences
- 7,315 unique pfam domains
- 6,362 unique KO functions

R = rhizosphere G = endosphere
P = *P. trichocarpa* B = *P. deltoides*

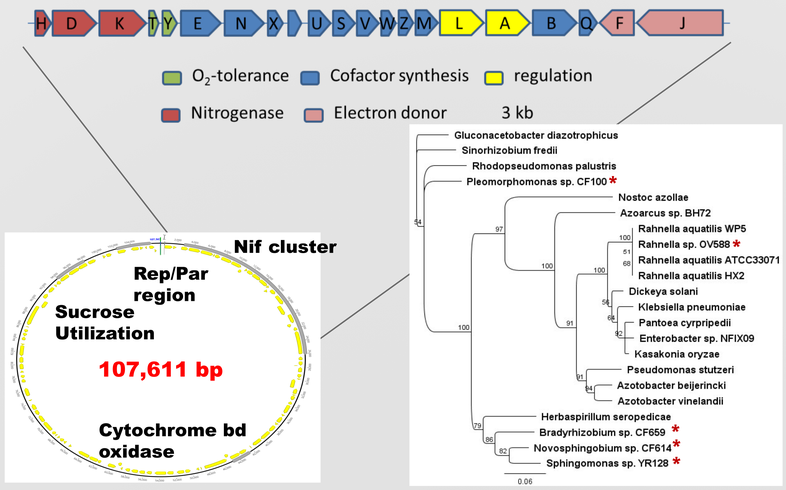
Top: Relative abundances (16S) of model community strains. Color shades are calculated by log10 transformation of relative abundance. Green means high abundance, white means low abundance. **Bottom:** Phylogenetic tree of *Populus* isolates generated from 16S rRNA sequence.

Rahnella sp. OV588 rhizobacterium

Isolated from *Populus trichocarpa* root tissue
γ-proteobacterium in the Yersiniaceae family

Plant growth promoting traits	Genome has been sequenced
Phosphate solubilization	5.4 Mb genome
Phytohormone production	52% G+C
Nitrogen fixation	4799 CDS
Quorum sensing	45 contigs
Plant colonization	4116 proteins with predicted functions

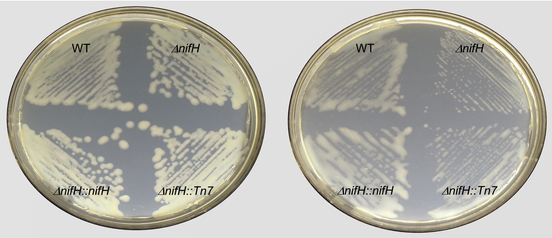
Nitrogenase cluster is encoded on plasmid



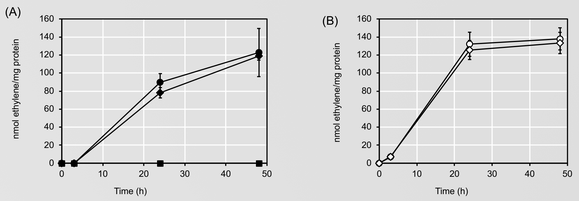
Left: Plasmid map constructed using Geneious software version 10.0.5 from *Rahnella aquatilis* OV588 EX24DRAFT_scaffold00016.16. **Right:** Phylogenetic tree based on NifH sequences constructed by neighbor-joining method. Scale represents substitutions/site. Indicates isolates from this project.

Rahnella catalyzes acetylene reduction *in vitro*

$$N_2 + 16ATP + 8H^+ + 8e^- \longrightarrow 2NH_3 + H_2 + 16ADP + P_i$$

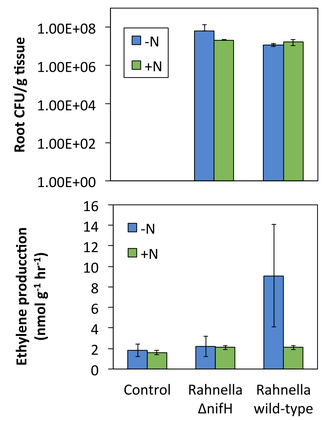


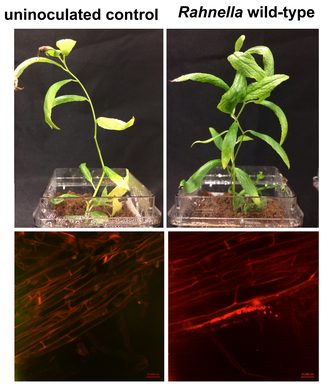
Above. Growth of OV588 WT, ΔnifH mutant, ΔnifH mutant complemented with wild-type nifH gene (ΔnifH::nifH) or vector control (ΔnifH::Tn7) strains on agar-plate supplemented with (A) or without (B) $(NH_4)_2SO_4$. Plates were incubated at 30 ˚C in the jar filled with nitrogen gas.



Above. Nitrogenase activity based on acetylene reduction assay from the cells scraped from agar-plate supplemented with (A) or without (B) $(NH_4)_2SO_4$. The results are the average of four biological replicates and standard deviation are shown as error bar. Symbols: closed circle, wild type OV588; closed diamond, Δ*nifH::nifH*; closed square, Δ*nifH::Tn7*; open circle, wild type OV588; open diamond, Δ*nifH::nifH*. Since there was no ethylene production from the ΔnifH mutant and ΔnifH::Tn7 from agar-plate supplemented with $(NH_4)_2SO4$, only the data from ΔnifH::Tn7 is shown in (A).

Rahnella catalyzes acetylene reduction in association with *Populus*

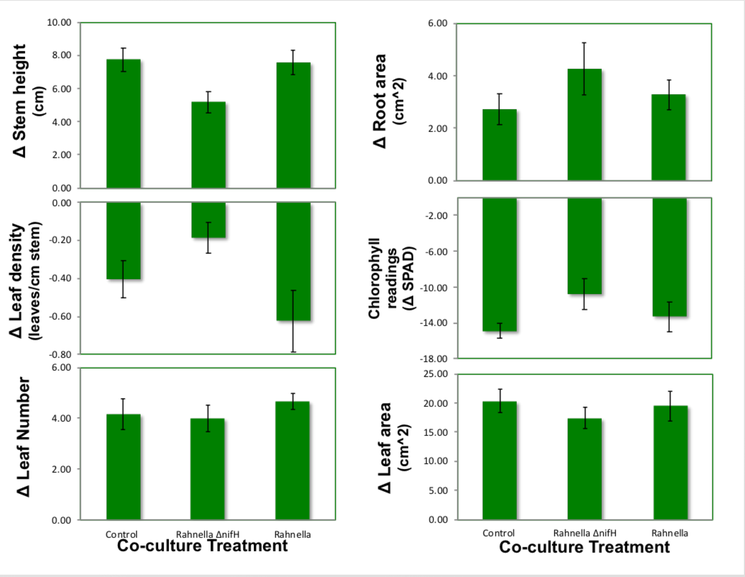




*Microscropic images Amber Bible & Jenny Morell - Falvey

Left Panel: Root colony formation units (CFU) and acetylene reduction assay results. **Right Panel:** Representative plants images after three weeks of growth in co-culture conditions with no added N. Bottom images show roots with mCherry modified *Rahnella* (right) and with uninoculated control (left).

Wild-type *Rahnella* benefits plant at low N conditions



Above: Panel of plant phenotypic measurements. Germ-free tissue culture plants were co-cultured with either dead autoclaved *Rahnella* wild-type (control), *Rahnella* Δ*nifH* mutant, or *Rahnella* wild-type. Plants were harvested after three weeks of growth in co-culture conditions with no added N.

Conclusions

Our research demonstrates that:

- **Reference synthetic communities can be reproducibly applied to *Populus trichocarpa* and *P. deltoides***
- **N2-fixing diazotroph bacteria were isolated from *Populus* and functionally characterized**
- **The resynthesis of the characterized diazotroph to axenic *Populus* benefited the plant on low N conditions**

References:

- **Timm, Collin M., et al. Abiotic Stresses Shift Belowground *Populus*-Associated Bacteria Toward a Core Stress Microbiome.*mSystems*3.1 (2018): e00070-17.**
- **Timm, Collin M., et al. Two poplar-associated bacterial isolates induce additive favorable responses in a constructed plant-microbiome system. *Frontiers in plant science*7 (2016): 497**
- **Timm, Collin M., et al. Metabolic functions of Pseudomonas fluorescens strains from *Populus deltoides* depend on rhizosphere or endosphere isolation compartment. *Frontiers in microbiology*6 (2015): 1118**

Acknowledgement:

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