Plant-Microbe Interfaces: Altered root metabolome composition impacts microbiome composition in *Populus PdKOR1* RNAi plants

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Abstract

In the present study, we examined the effect of altered carbon partitioning and allocation in the *Populus* host due to interactions with individual beneficial microbes as well as on the overall root endosphere and rhizosphere associated microbiome. The altered secondary metabolism host type used in this study are transgenic *Populus PdKOR1* RNAi plants that are downregulated in an endo-β-1,4-glucanase gene family member. Gas chromatography-mass spectrometry profiles of *PdKOR1* plants showed a higher phenolic and salicylic acid content, and reduced lignin, sugars, shikimic acid and maleic acid content relative to non-transgenic control. Co-culture with the fungal mutualist, *Laccaria bicolor*, showed enhanced mycorrhization rate and improved biomass production in *PdKOR* plants (Kalluri et al. 2016). This suggested strong potential for impact on the broader microbial community that the plant interacts with in field settings. In contrast, the colonization rate of a previously characterized Gammaproteobacterial isolate, *Pantoea* YR343, was lower in *PdKOR1* RNAi plants. To test whether altered root metabolome has an effect on the microbiome associated with roots under fields settings, we collected root samples from independent ramets of field-grown *PdKOR1* RNAi and control plants and performed Illumina MiSeq 16S rRNA gene sequencing. Bacterial community composition, as measured by Bray-Curtis dissimilarity, differed between *PdKOR1* RNAi and control rhizospheres and roots. *Actinobacteria*, and the family *Micromonosporaceae*, were significantly more abundant, whereas *Nitrospirae* were reduced in *PdKOR1* RNAi plant rhizosphere. These findings from single isolate co-culture experiments as well as field-based microbiome analyses show the relevance of host carbon partitioning and metabolome composition, including phenolic, sugar, amino acid and fatty-acid composition, on concomitant alterations in root-associated microbial communities. We are currently conducting metagenomics of leaf, stem and root and soil samples to capture the genetic diversity of the microbial communities (bacterial and fungal) associated with specific plant tissue type/niche, and examining the differential molecular pathways underlying the differential association of microbes via RNA-Seq analysis and microbial isolate sequencing approaches. In conclusion, our study shows the significance of plant metabolome composition on shaping the associated microbiome and is prompting new hypotheses and experiments that will address the cascading effects of host genotype, root tissue environment, root exudate composition on interactions with soil microbiome.

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Experimental context

Co-culture experiments under controlled greenhouse conditions

Microbial community profiling under field conditions

Sampling of fine roots in the field for metabolome and microbiome profiling

Characterization of root metabolome and proteome, and microbial colonization under lab conditions

Results

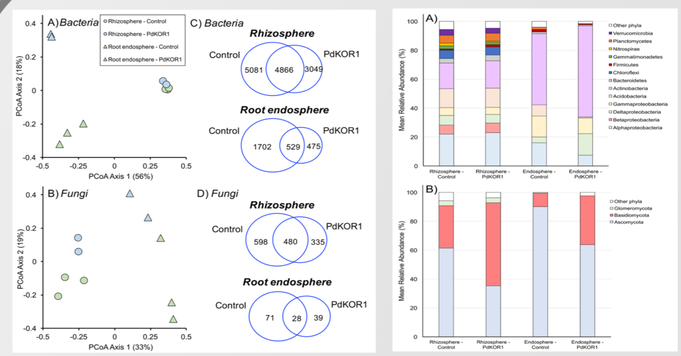


Fig. 2. Principal coordinates analysis (PCoA) of 16S rRNA-based bacterial (Panel A) and ITS2 rRNA-based fungal (Panel B) community composition within rhizosphere and root endosphere habitats in vector control and *PdKOR1* RNAi plants. Venn diagrams of unique and shared OTUs across rhizosphere and root endosphere habitats in control and RNAi plants for bacteria (Panel C) and fungi (Panel D). Circle size in panels C-D is scaled based on OTU number within control or RNAi plants in a habitat, but are not scaled for comparison across rhizosphere and endospheres. Numbers in circles, or in overlapping sections, represent the number of OTUs in that category.

Fig. 3. Mean relative abundance of dominant (≥ 1.0%) bacterial phyla and subphyla for Proteobacteria (Panel A) and fungal phyla (Panel B) among rhizosphere soils and root endospheres within controls and *PdKOR1* RNAi plants.

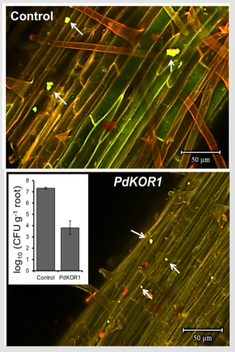


Fig. 4. Confocal imaging of root colonization in *Populus deltoides* control and *PdKOR1* with *Pantoea* YR343-GFP. Colonization of control appears in the form of small aggregates, typical of YR343, whereas *PdKOR1* colonization has individual cells present. Arrows indicate *Pantoea* YR343-GFP attached to the root surface. Colonization of *Populus deltoides* control and *PdKOR1* RNAi plants with *Pantoea* YR343-GFP showed a decrease in root colonization (measured as CFUs per gram of root) in RNAi lines as compared to the control lines (P = 0.007).

Metabolite	PdKOR/Control	
[Retention time (min); Key m/z]	Fold change	p-value
20.68 171 caffeoyl conjugate	112.06	0.006
23.59 179 caffeoyl-shikimate conjugate	66.66	0.005
19.72 171 331 coumaroyl conjugate	42.55	0.020
20.00 171 331 coumaroyl conjugate	34.51	0.006
20.82 375 597 coumaroyl conjugate	25.43	0.017
21.17 171 caffeoyl shikimate conjugate	21.95	0.008
20.91 171 caffeoyl-shikimate conjugate	20.21	0.005
20.23 171 331 coumaroyl conjugate	19.20	0.038

Fig. 5. Metabolite profiling reveals an overrepresentation of phenolic metabolites in roots of field-grown *PdKOR1* RNAi roots.

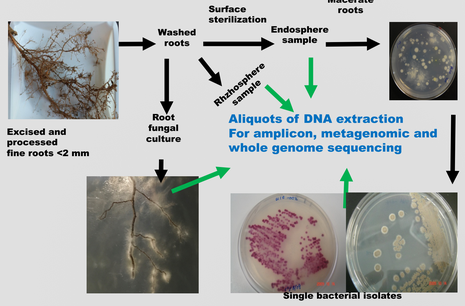
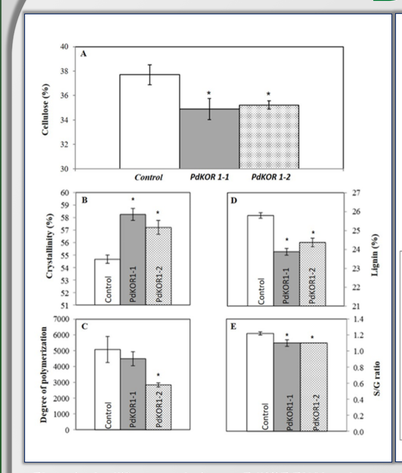
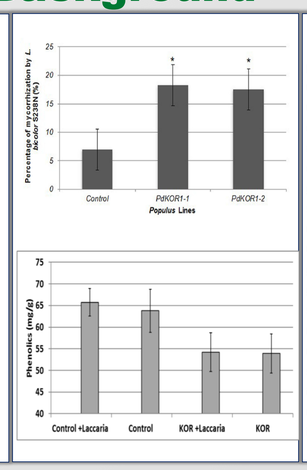


Fig. 6. A workflow for sample processing, microbial isolation, and sequencing.

Background



Phenotypic differences between *PdKOR* RNAi and control lines



Enhanced colonization rate and decreased phenolic content in *PdKOR* roots

	PdKOR1-1	PdKOR1-2	PdKOR2-1	PdKOR2-2
putrescine	6.70	3.21	1.59	1.38
unknown (10.03 min, 159)	3.65	6.12	2.86	2.73
unknown (11.98 min, 232 449 359)	2.34	5.72	2.42	2.40
cis-aconitic acid	2.19	2.54	1.47	1.86
asparagine	2.12	6.08	2.99	2.82
oxo-proline	1.99	2.13	1.26	1.39
glycerol-t3-phosphate	1.96	1.58	1.49	1.39
sucrose	1.71	1.18	1.10	1.00
unknown	1.58	1.73	1.42	1.26
GABA	1.57	1.52	1.21	1.11
glucose	1.20	0.67	0.38	0.37
galactose	1.19	0.59	0.35	0.37
salicin	0.87	0.82	1.04	0.56
raffinose	0.82	0.69	0.59	0.28
unknown (11.16 min, 306)	0.52	0.39	0.40	0.33
shikimic acid	0.50	0.32	0.23	0.23
threonic acid	0.34	0.27	0.16	0.21
maleic acid	0.33	0.31	0.23	0.21
fructose	0.30	0.16	0.14	0.13

Differential representation of metabolites in *PdKOR* relative to the control

Fig. 1. Down-regulation of *KORRIGAN*-like endo-β-1,4-glucanase genes impacts carbon partitioning, mycorrhizal colonization and biomass chemistry in *Populus* (Kalluri et al. 2016).

Conclusions and Ongoing Work

- **Our insights from both field and greenhouse experimental conditions demonstrate interconnections among plant genotype, cellulose biosynthesis, cell wall properties, metabolome and microbial community composition.**
- **Specifically, constitutive down-regulation of *PdKOR1* and accompanying cell wall modifications affect secondary C metabolism and both bacterial and fungal community structure within rhizospheres and root tissues in field-grown *Populus*.**
- **More expansive efforts to characterize the microbiomes of a suite of additional host transgenic lines contrasted in cell wall and metabolome changes in the field along with lab-and greenhouse-based validation experiments should help clarify whether microbial community differences are a result of metabolomic changes, or due to other phenotypic traits disrupted by *PdKOR1* down-regulation.**
- **A detailed study is underway as part of a JGI-CSP project (Kalluri et al. 2018) JGI for metagenomics analysis and genome sequencing of microbial isolates and profiles. This presents a unique resource to ask the question as to whether the genetically-driven differential root metabolite composition translates into a unique root exudate profile which drives differences in colonization of soil microbial populations.**
- **A knowledge base that captures the spectrum of functional implications of biomass chemistry improvement efforts is crucial for meeting the goals of sustainable bioenergy crop production and management.**

References:

Kalluri et al. 2016. Frontiers in Plant Sciences 2016. doi: 10.3389/fpls.2016.01455.

Kalluri et al. 2018. FY18 DOE Joint Genome Institute Community Sequencing Project.

Acknowledgement:

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