# Plant-Microbe Interfaces: Characterization of natural products from the *Populus* microbiome

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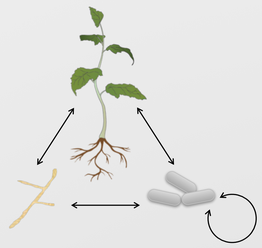
## Objective



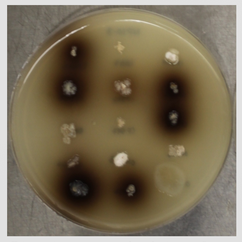
**The *Populus* root microbiome is an incredibly diverse community, comprising organisms from across plant, animal, oomycete, fungal, viral, archaeal and bacterial taxa. Bacteria from the soil are known to harbor many gene clusters encoding complex natural products that can act as signaling molecules, antibiotics, and antifungals. We set out to characterize the natural product potential of bacteria from a plant root community in order to understand its biosynthetic diversity as well as begin to determine keystone members and associated molecules that regulate community structure and plant health.**

## Approach

**The model plant system used was *Populus*, the first fully genome-sequenced tree species having an already well-characterized root metagenome.**

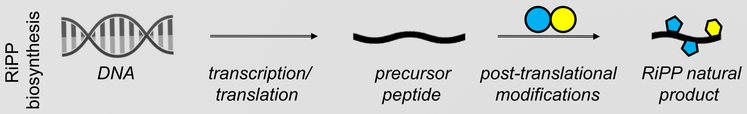
**We first considered metagenomic samples collected from the roots of P. deltoides and examined the overall bacterial diversity and natural product variety, comparing to other plant and human microbiomes to show the predicted species and natural product richness. The diversity of bacteria in the plant microbiome is greater than the well-studied human gut microbiome, and the organisms within this community have greater biosynthetic potential as well.**

**We next utilized the fully sequenced genomes of over 337 bacterial isolates, representing the four major bacterial phyla in the metagenome, connecting molecules to genomes and surveying the overall natural product potential. While some species harbor greater numbers of clusters, especially Actinobacteria of the genus *Streptomyces*, we found over 10 clusters per organism on average, with over 4000 predicted clusters. Comparison to known natural product gene clusters revealed that only 1% of clusters produced an already-characterized secondary metabolite, revealing the great potential to discover compounds with novel structures and possibly novel activities.**



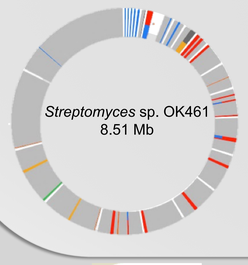
*Streptomyces* isolates from the *Populus* microbiome have diverse colony morphology, and equally diverse natural product clusters.

**About 15% of the predicted clusters could not be connected to known natural products classes, revealing the potential to discover structurally novel metabolites. Of the remaining clusters, many grouped within classes known to produce molecules with antibiotic or antifungal properties. Ribosomally synthesized and post-translationally modified peptide natural products were both prevalent in the collection and divergent from previously characterized molecules. These natural products, which are peptides that have been modified by additional enzymes, were the most abundant class of natural product identified, being more common than even nonribosomal peptide and polyketide clusters.**




**While the diversity and richness of natural product gene clusters within the genome-sequenced fraction of the *Populus* microbiome reveals an additional layer of complexity in the community, the presence of a gene cluster does not necessarily mean that the compound will be produced.**

**We determined if genome-sequenced *Streptomyces* isolates were capable of producing compounds with antifungal and antibiotic activity under laboratory conditions. Four *Streptomyces* isolates each contain an identical lasso peptide gene cluster with known Gram-positive antibacterial activity. Both isolates inhibited the growth of Gram-positive isolates, and methanolic extracts containing the lasso peptide replicate this activity.**

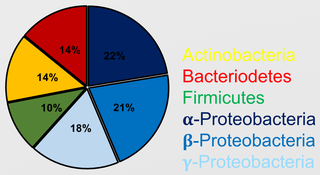






PRISM representation of the genome of *Streptomyces* sp. OK807, with colored bars highlighting sections of the genome containing biosynthetic gene clusters.

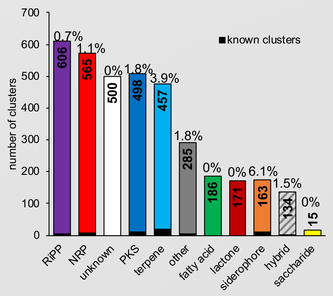
## A diverse microbiome with a diverse natural product potential

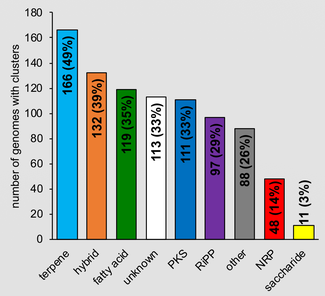



**Phylogenomic distribution of sequenced bacteria from Populus roots**

### Biosynthetic gene clusters (BGCs) discovered through genome mining of 339 sequenced bacterial isolates

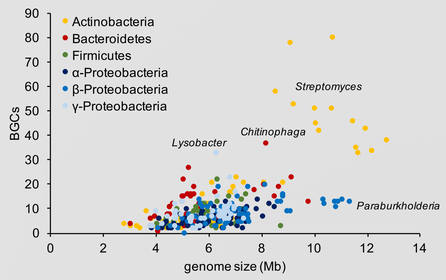
- 3409 BGCs predicted using antiSMASH genome mining software
- >10 BGCs/organism
- Larger diversity of natural products and more BGCs/organism than in the human gut microbiome



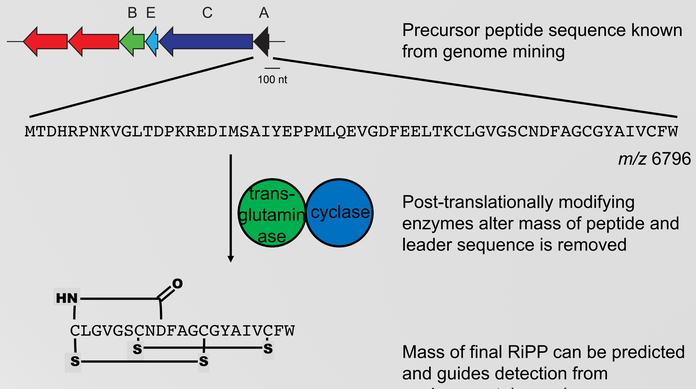




- 98.9% of clusters encode an uncharacterized natural product
- Great potential to discover completely novel natural products

### *Streptomyces* genomes are especially dense in BGCs




### Structure prediction of RiPP natural products




Precursor peptide sequence known from genome mining

Post-translationally modifying enzymes alter mass of peptide and leader sequence is removed

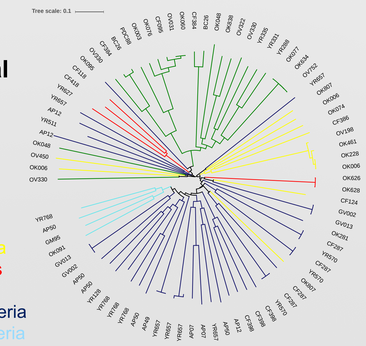
Mass of final RiPP can be predicted and guides detection from environmental samples

## Lasso peptides from the *Populus* microbiome

**70 Lasso peptide clusters were identified in 46 genomes, making this RiPP one of the more prevalent natural product classes discovered in the *Populus* microbiome**

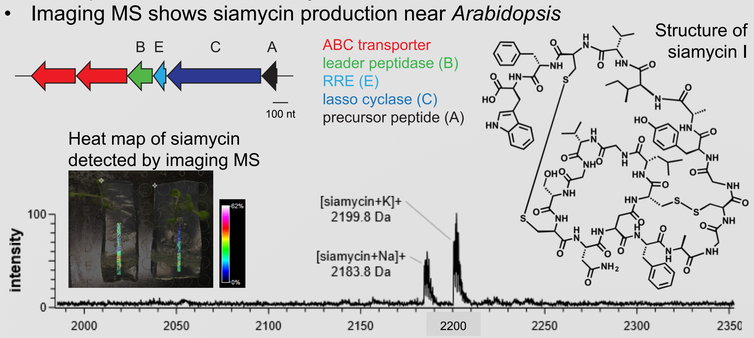
### Diversity of lasso peptide sequences from *Populus* bacterial symbionts

- Gene clusters present in all four bacterial phyla
- Most are class II lasso peptides, with no disulfide bonds
- 14-38 amino acids long




### The siamycin I gene cluster has been found in four *Streptomyces* isolates

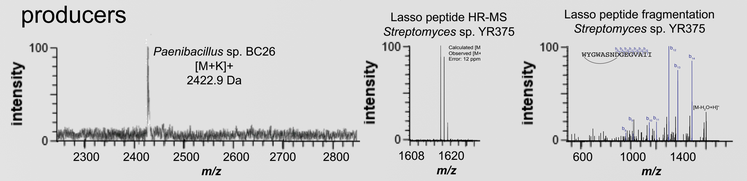
- Class I lasso peptide with 21 amino acids and a Cys1-Asp9 isopeptide bond closing the macrocyle
- Production observed in all four organisms using MALDI-TOF-MS
- Gram positive antibiotic activity confirmed
- Imaging MS shows siamycin production near *Arabidopsis*




MALDI-TOF-MS spectrum confirming siamycin production from *Streptomyces* sp. OK461

### Novel lasso peptides are produced by members of the *Populus* microbiome

- Lasso peptide predicted masses were used to guide discovery
- Unique lassos from all four phyla were detected, in at least 18/46 producers




## Conclusions

**Bacteria in the *Populus* microbiome:**

- **Encode many natural product gene clusters**
- **May produce many novel natural products**

**Complex molecules from bacteria in the *Populus* microbiome:**

- **Enable interspecies signaling and communication**
- **Shape community structure**
- **Influence plant health**

**Lasso peptides from bacteria in the *Populus* microbiome:**

- **Are produced under laboratory conditions**
- **Have antibiotic activity**

**References:**

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Skinnider MA, Dejong CA, Magarvey NA, et al. *Nucleic Acids Res* **2015,** *43* (20), 9645-9662.
Weber, T.; Blin, K.; Medema, M. H., et al. *Nucleic Acids Res* **2015,** *43* (W1), W237-W243.

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