Plant-Microbe Interfaces: Characterization of IAA biosynthesis pathways in *Populus*-associated microbes

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Project Goals: The goal of the PMI SFA is to understand the genome-dependent molecular and cellular events involved in establishing and maintaining beneficial interactions between plants and microbes. *Populus* and its associated microbial community serve as the experimental system for understanding how these molecular events manifest themselves within the spatially, structurally, and temporally complex scales of natural systems. To achieve this goal, we focus on 1) characterizing host and environmental drivers for diversity and function in the *Populus* microbiome, 2) utilizing microbial model system studies to elucidate *Populus*-microbial interactions at the molecular level and dissecting the signals and pathways responsible for initiating and maintaining microbial relationships and 3) develop metabolic and genomic modeling of these interactions to aid in interpreting the molecular mechanisms shaping the *Populus*-microbial interface.

Populus deltoides (poplar) hosts a diverse microbiome that influences its growth and productivity. Many plant-associated bacteria have the ability to produce phytohormones, such as indole-3-acetic acid (IAA). However, elucidating the pathways associated with secondary metabolite production in microorganisms is an ongoing challenge. Multiple IAA biosynthetic pathways have been described in microbes, most of which require the precursor tryptophan. The tryptophan-dependent pathways include the indole-3-acetonitrile (IAN) pathway, the indole-3-acetamide (IAM) pathway, the tryptophan side-chain oxidase (TSO) pathway, the indole-3-pyruvate (IPA) pathway, and the tryptamine pathway. We are pursuing the use of genetic knockouts and –omics measurements along with cell-free metabolic engineering coupled with bioinformatic searches of genome databases and protein-ligand docking simulations in order to identify the proteins most likely to be involved in a metabolic pathway. In particular, genomic

analysis was used to predict that *Pantoea* sp. YR343 synthesizes IAA using the indole-3-pyruvate (IPA) pathway. This prediction was tested using a combination of proteomics, metabolomics and genetics. To better understand IAA biosynthesis and the effects of IAA exposure on cell physiology, we characterized proteomes of *Pantoea* sp. YR343 grown in the presence of tryptophan or IAA. These data indicate that indole-3-pyruvate decarboxylase (IpdC), a key enzyme in the IPA pathway, is upregulated in the presence of tryptophan and IAA. Metabolite profiles of wildtype cells showed the production of IPA, IAA, and tryptophol, which is also consistent with an active IPA pathway. Finally, we constructed a mutant in *Pantoea* sp. YR343 in which the *ipdC* gene was deleted. This mutant was unable to produce tryptophol, consistent with a loss of IpdC activity, but was still able to produce IAA (20% of wildtype levels). This result suggests the possibility of an alternate pathway or the production of IAA by a non-enzymatic route. To examine this possibility and to aid in the assignment of candidate enzymes in the pathways, we employed protein-ligand docking simulations. The resulting computationally predicted set of enzymes were then expressed and are being tested in cell free systems for their ability to produce IAA.

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