Functional assignment of ligand specificities for Pseudomonas transport proteins

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Project Goals: Symbiosis between soil bacteria, mycorrhizal fungi and tree roots leads to coordinated resource exchange and enhanced productivity and resiliency in forest ecosystems. Although nutrient cycling is a key feature of these interactions, very little is known about the molecular mechanisms underpinning this process or the impact on soil community structure. The Argonne "Environment Sensing and Response" Scientific Focus Area (ESR-SFA) program aims to elucidate plant-microbial interactions between *Populus tremuloides* (Quaking aspen), the ectomycorrhizal fungus *Laccaria biocolor* and the bacterium *Pseudomonas fluorescens,* and to understand how the fungal and bacterial symbionts influence plant capture, partitioning, and allocation of carbon (C) under nutrient limitation stresses.

Recently, a machine learning approach using sequenced Pseudomonad genomes coupled with outputs of metabolic and transportomic computational models revealed that the molecular mechanisms most predictive for ecological role of *Pseudomonas* in the rhizosphere is the ability to sense and manipulate its environment via its transmembrane transport capacity (*i. e.* the transportome) [Larsen et al., 2015]. However, the high number of transporter systems in *Pseudomonas* and the lack of specific information on the transported ligands limits the accuracy of our computational approaches and hinders our ability to experimentally validate specific predictions for mechanisms of plant-microbe interactions.

To address this limitation, we functionally characterized groups of high-affinity nutrient transporters belonging to the ATP Binding Cassette (ABC) and Tripartite ATP-independent Periplasmic (TRAP) transporters. Both systems utilize solute binding proteins (SBPs) to deliver substrates to transmembrane complexes for transport into the cytoplasm. Two sets of SBPs from plant-associated *Pseudomonas* have been characterized *in vitro*: organosulfur compound-binding SBPs and SBPs for simple carbon sources such as monosaccharide and dicarboxylic acids.

A high-throughput screening procedure was used to evaluate ligand binding activity through a fluorescence assay registering increased thermal stabilization. This approach is widely used for ligand identification and can integrate multiple protein targets and large ligand screening libraries. The resulting qualitative rankings of protein-ligand interactions were subsequently validated and quantified by determining affinity constants via Isothermal Titration Calorimetry.

We report protein-ligand interactions for eight SBPs binding organosulfur compounds, including two previously unknown binding activities. These activities expand the range of small molecules recognized by the methionine ABC transporter family and provide additional insight into the transport and metabolic capabilities of Pseudomonads [Zerbs et al., *in press*]. We also report protein-ligand interactions for eight SBPs binding monosaccharides and dicarboxylic acids—comprised of six ABC-derived proteins and two TRAP-derived proteins. Interestingly, several SBPs bound multiple ligands (e.g. L-arabinose and D-galactose, galacturonic and glucuronic acids) with similar affinities in the micro- and submicromolar range.

To investigate whether these binding profiles accurately reflected ligand transport, we deleted genes encoding the SBP and cognate membrane-associated transporter from the genome. Knockout (KO) mutants were grown with various ligands as sole carbon source to determine *in vivo* effects of transporter removal. Six transporter systems were successfully deleted from the *P. fluorescens* SBW25 chromosome, using a DNA recombineering approach. Removal of transporters for C4-dicarboxylic acids and uronic acids had no observable effect on growth, suggesting that SBW25 has additional transporters for these compounds. Removal of the transporter associated with glucose-binding SBP had a moderate growth defect, confirming its physiological role in glucose transport in a context where other glucose transporters are operating. Of the two putative arabinose-galatose transporters, one preferentially transports arabinose while the other preferentially transports galactose. A double KO of both transporters showed reduced growth on both sugars relative to either single mutant, indicating that while each transporter has a preference for arabinose or galactose, it also has a secondary role in transporting the other sugar. These results support the functional assignments obtained from biochemical assays, and suggest that transport specificity is also determined by other factors downstream of the SBP. Finally, these functional assignment will allow us to test experimentally the predicted role of arabinose transport in plant growth promotion (see S. Shinde poster).