A Mycorrhizal Helper Bacteria Increases Mycorrhization in Aspen Seedlings by Modulating Expression of Defense Response Genes in Roots

Peter E. Larsen[1,2*] (plarsen@anl.gov), Shalaka Shinde[1], Sarah Zerbs[1], Frank R. Collart[1], Jonathan Cumming[3], and **Philippe Noirot**[1].

[1] Argonne National Laboratory, Biosciences Division, IL; [2] University of Illinois at Chicago, Department of Bioengineering, Chicago IL; [3] West Virginia University, Biology Department, Morgantown

Project Goals: Use transcriptomic analysis of a laboratory rhizosphere community to identify the molecular mechanisms in aspen roots by which the mycorrhizal helper bacteria SBW25 induces increased mycorrhization of aspen seedling roots by the ectomycorrhizal fungi Laccaria.

Beneficial rhizosphere communities of symbiotic fungi and bacteria protect plants from a variety of biotic and abiotic stresses. In return, plants provide photosynthetically fixed carbon to rhizosphere community members, making these subsurface communities not only crucial for the health and stability of terrestrial ecosystems, but also important components of the carbon cycle. One of the beneficial roles bacteria can play in the rhizosphere community is that of Mycorrhizal Helper Bacteria (MHB). MHB facilitate positive symbiotic interactions between sol fungi and plant roots. While the positive effects of MHB on mycorrhizal interactions have been well characterized, the specific molecular mechanisms by which MHB enhance mycorrhizal interactions are less well established. We have developed a sand-pot, tripartite laboratory community of aspen seedlings, ectomycorrhizal fungi *Laccaria bicolor* (Laccaria), and the Plant Growth Promoting (PGP) [1] bacteria *Pseudomonas fluorescens* SBW25 (SBW25), suitable for HTP omics analysis. Here, we utilize this laboratory community model system to propose the specific molecular mechanisms in aspen roots that drive MHB activity.

Our experimental design is comprised of four biological conditions: aspen seedlings, aspen with Laccaria, aspen with SBW25, and aspen with Laccaria and SBW25. Rhizosphere communities were cultured for 63 days, at which time aspen seedling phenotype data and community transcriptomes were collected. We found that the presence of SBW25 significantly increased mycorrhization of aspen seedling roots by Laccaria by almost 2-fold, demonstrating that SBW25 is a MHB under our experimental conditions. The analysis of community transcriptomic data revealed that both Laccaria and SBW25 mRNA were detectable at statistically significant levels in the sand pot rhizosphere after 63 days. Using computational and modeling methods we have previously developed for the analysis of rhizosphere community interactions [2,3], we identified six clusters of co-regulated genes is aspen seedling roots across biological conditions. One gene cluster, significantly enriched for anti-fungal defense response genes, was found to be highly up-regulated when Laccaria is present alone, but down-regulated when both Laccaria and SBW25 are present. This suggests that the inhibition by SBW25 of fungal defense-response genes in aspen roots is a key component to SBW25's MHB activity. However, this finding does not implicate the specific regulatory mechanisms by which fungal defense response genes are controlled.

By considering the expression pattern of each gene cluster as a conditional statement, it becomes possible to arrange the clusters of differentially-expressed co-regulated genes as a logic circuit (**Figure 1**), highlighting potentially causal relationships between rhizosphere community composition, patterns of gene regulation, and observed MHB activity. The mechanism that enables MHB activity in this predicted network is a XOR logic gate that down-regulates global-regulation genes when both Laccaria and SBW25 are present in the rhizosphere community, but not when Laccaria or SBW25 are present individually. Statistically significant enrichment for genes with the annotation 'pollen recognition' indicate excellent

candidates for the molecular mechanism of the predicted XOR gate that down-regulates either the global gene regulation or the fungal defense response gene cluster in the gene regulation logic circuit diagram.

The results of this analysis propose three key findings: (i) SBW25 is a MHB under our experimental conditions, (ii) increased mycorrhization by Laccaria when SBW25 is also present occurs in response to the down-regulation of aspen root fungal-defense response genes, and (iii) the molecular mechanisms by which aspen roots detect the rhizosphere community involve sensors with homology to 'pollen detection' sensors. Future analyses will further characterize these sensors and identify the specific ligands present in the rhizosphere that aspen roots used to collect information about the composition of the rhizosphere community.
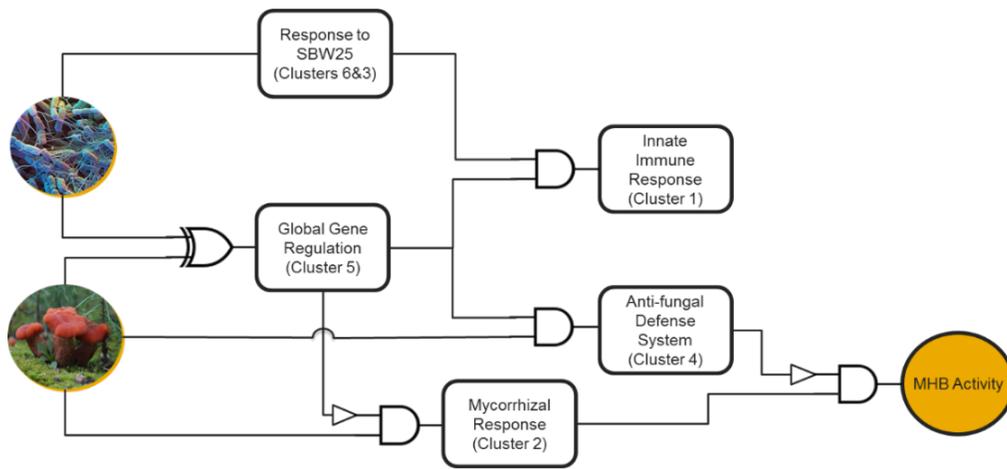
Figure 1. Predicted aspen root gene regulatory network for MHB activity as a logic circuit diagram. The proposed regulatory interactions between co-expressed gene clusters is represented as a logic circuit diagram. In the network, circles to left indicate presence or absence of Laccaria (red mushrooms) or SBW25 (blue microscopy image). Rectangles are co-regulated gene clusters identified by K-means clustering and gene cluster function descriptions are taken from statistically enriched functional annotations within gene clusters. An edge between nodes indicate a predicted causal relationships between gene clusters, inferred from observed patterns of gene regulation.

References

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