Principles of Fungal Metabolism, Growth and Bacterial Interactions.

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Project Goals: The goals of this project is to elucidate fundamental principles of species interactions using hybrid machine learning/simulation models of *fungal-bacterial interactions and dynamics*. These hybrid data-analytic/simulation models are being used to carry out virtual experiments and develop fundamental understanding of the interactions between fungi and bacteria, specifically the mycorrhizal fungus *Laccaria bicolor* and the helper bacteria closely related to *Pseudomonas fluorescens.* At the same time, we carry out experiments aimed at developing and testing quantitative assays to measure the same interactions, and whose data will inform the our view of biology. We are:

- Evaluating the impacts of (1) thiamine and phenazines and (2) trehalose, produced respectively by *P. fluorescens* and *L. bicolor* on the metabolisms of each other. Metabolic exchange is an emerging theme in bacterial-fungal and bacterial-bacterial interactions.
- Characterizing *L. bicolor* -stimulated chemotaxis of pseudomonas by coupling trehalose signaling and metabolism to chemotaxis of pseudomonads.
- Experimentally investigating (1) pseudomonas chemotaxis in response to *L. bicolor* produced metabolites, and growth of *L. bicolor* in response to metabolites secreted by pseudomonads.

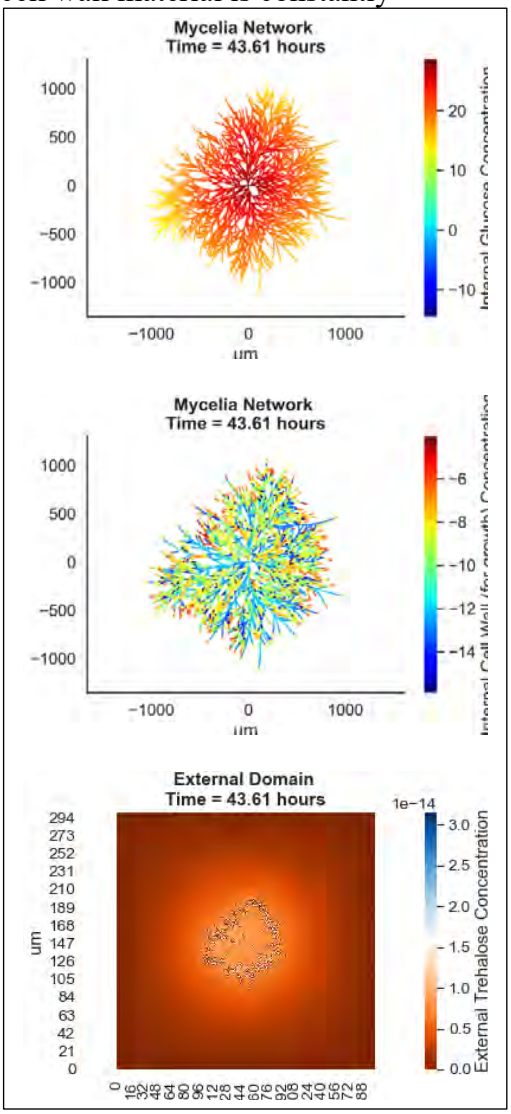
Abstract: The exchange of metabolites between microbes is an emergent property that evolves because the exchanged metabolites allow for increased growth of both species by reducing the thermodynamic cost of growth. Instead of each species producing every metabolite needed, metabolite exchange allows each microbe to specialize and efficiently produce a metabolite, such as trehalose, in exchange for one that it cannot produce as cheaply, such as thiamine. In economics this is known as Ricardo's principle of comparative advantage [1]. In order to evaluate the benefits of such microbial trade, physics-based models are needed that are capable of modeling the thermodynamic costs and benefits. The long-term goal is to understand fundamental principles of fungal-bacterial interactions through physics-based models of metabolism, protein expression and gene expression and to couple these models to the mycelial growth and bacterial chemotaxis.

The parameters for the ordinary differential equations (ODEs) needed to model the dynamics of metabolism are obtained by exploiting the natural selection principle that organisms that have the highest entropy production rates as a group will outcompete species with lower entropy production rates. Because we are using detailed models, entropy production includes growth, maintenance and catabolism. Another way of stating the entropy production principle is that the organisms that grow the fastest and most efficient will out compete others with slower and less efficient metabolisms. This perspective of metabolism subsumes many ecological concepts such as the red queen hypothesis and the black queen hypothesis. The maximum entropy production principle has a form that can be explicitly derived at the scale of metabolism.

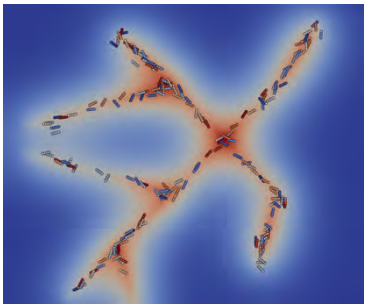
Our *Laccaria bicolor* metabolic model currently includes all reactions of central metabolism, synthesis of all 20 proteogenic amino-acids, synthesis of cofactors, synthesis of ATP, GTP, UTP, CTP and TTP and all deoxy-nucleic acid forms, synthesis of generic proteins, synthesis of fatty acids, sterols, synthesis of generic DNA and RNA strands and growth on minimal media of

glucose, ammonia, sulfate, and phosphate. Regulation of the metabolic activity is carried out by a method that combines reinforcement learning of control with statistical thermodynamics and metabolic control analysis, a branch of control theory [2].

A picture is emerging of natural oscillations within metabolism that are related to the cell cycle and circadian rhythms. In this picture, the NADPH/NADP ratio oscillates between high and low values, driving alternately DNA and fatty acid synthesis (high NADPH/NADP) and protein and RNA synthesis (low NADPH/NADP). Throughout the cycle, cell wall material is constantly produced, leading to an ever growing mycelium.



Concurrently, we are developing structural models of the fungal mycelial growth. The mycelial models (right) take in glucose (top right), convert the glucose to cell wall precursors which are actively transported through the fungal hyphae (middle right), and produce chemo-attractants which are exported and diffuse away in the external environment (bottom right). The internal metabolism of each hyphal segment currently uses a system of coupled Michealis-Menten systems along with equations for diffusive and active transport across hyphal septa. In the coming year, we expect to replace the Michaelis-Menten models with full metabolic models discussed above, allowing us to study the details of the thermodynamic benefits of metabolic exchanges with soil bacteria.



Also concurrently, we are developing models of chemotactic Pseudomonas species that interact with the fungal mycelia described above (left). The bacteria consist of subcellular element models to describe the structure. The bacteria run, flick and then reverse directions in order to navigate toward nutrition sources. The frequency of reversing the direction of motion is controlled by an internal clock. The bacteria have difficulty moving in solid media such as agar (or dehydrated soil) but the water excreted by the fungi due to metabolic activity provides a highway for the bacteria.

References:

[1] J. Tasoff, M. T. Mee, and H. H. Wang, "An Economic Framework of Microbial Trade," *PLoS One,* vol. 10, no. 7, p. e0132907, 2015, doi: 10.1371/journal.pone.0132907.

[2] S. Britton, M. Alber, and W. R. Cannon, "Enzyme activities predicted by metabolite concentrations and solvent capacity in the cell," *J R Soc Interface,* vol. 17, no. 171, p. 20200656, Oct 2020, doi: 10.1098/rsif.2020.0656.

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