Spatiotemporal mapping of the origin of linolenic acid in signaling transduction cascades during leaf deconstruction

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This early career research project is dedicated to achieving transformative molecular-level insights into microbial lignocellulose deconstruction through the comprehensive and informative view of underlying biological pathways provided by the integration of spatiotemporal multi-omic measurements (i.e., proteomics, metabolomics, and lipidomics). A focus of this project is to uncover the mechanisms that drive cooperative fungal-bacterial interactions that result in the degradation of lignocellulosic plant material in the leaf-cutter ant fungal garden ecosystem. This approach will provide the knowledge needed for a predictive systems-level understanding of the fungal-bacterial metabolic and signaling interactions that occur during cellulose deconstruction in an efficient, natural ecosystem.

Naturally evolved microbial systems that are capable of efficient deconstruction of plant cell wall biomass exist. Biomass deconstruction in these natural communities is often dependent on bacterial-fungal symbiosis, yet the molecular underpinnings of these interactions are poorly understood. An excellent example of such a system is the leaf-cutter ant fungal garden ecosystem, which employs inter-kingdom interactions to liberate energy rich carbohydrates from plant lignocellulose biomass. Unfortunately, the microbial community dynamics of the leaf-cutter ant fungal garden ecosystem are a challenge to assess because of the high heterogeneity of species composition and phenotype occurring across space and time during plant biomass deconstruction.

To understand how the fungal garden is able to degrade plant matter with such efficiency, it is necessary to study the metabolic interactions and biochemical pathways utilized by its microorganisms in each microscopic region of the fungal garden. This research will accomplish that with novel microscale metabolomics, lipidomics, and proteomics approaches that can analyze very small samples, providing detailed information on the location and function of fungal and bacterial molecules. In this initial study, we evaluated the lipidomic differences between the leaves feeding the gardens and spatially-resolve regions of the fungus garden at initial to advanced stages of leaf degradation. Lipids containing alpha-linolenic acid (18:3) from the leaves were enriched in the top of the gardens, where lysophosphatidylcholines (LPC) provided evidence for phospholipase activity and 18:3 signaling which adversely impact fungus health and plant biomass degradation.

When leaves are wounded, the polypeptide systemin is emitted from the damaged cells into the apoplast, signaling the liberation of 18:3 from plant membrane lipids into the cells. 18:3

begins the defense pathway by being converted to 12-oxophytodienoic acid and then through beta-oxidation is converted into jasmonic acid. The main defense mechanism in these leaves is jasmonic acid, and 18:3 is crucial to its synthesis. Despite extensive study of the cascade of molecular events in response to plant wounding, there is limited knowledge on the contribution and fate of individual membrane lipids, and specificity of phospholipase enzymes in this process. Which is the reason why we still do not know the exact origin of linolenic acid in the signaling pathway. To visualize lipid composition at wound sites with micrometer-scale resolution, we used two complementary matrix-assisted laser desorption/ionization (MALDI) mass spectrometry imaging (MSI) platforms. We performed MALDI-Fourier transform ion cyclotron resonance (FTICR)-MSI and MALDI-trapped ion mobility spectrometry time-of-flight (timsTOF)-MSI experiments across wounded leaf sections of *Solanum lycopersicum* which is a model system for studying plant defense signaling. With concurrent mapping of phospholipids with 18:3 fatty acid composition and lysolipid spatial behavior we obtained insight into the possible origin of linolenic acid in the wounding process. Among lysolipids, LPC species strongly co-localize with the injured zone of wounded leaflets in all bioreplicates. We observed the highest spatial correlation between LPC (16:0): LPC (18:3), LPC (16:0): LPC (18:2) and LPC (16:0): LPC (18:0) ion image pairs, while lower correlation is observed between individual 18C LPCs.

Here we explore how lipid levels change in leaves of dicot plants during microbial degradation and mechanical wounding. Both studies suggest that linolenic acids are predominantly released from phosphatidylcholines (PCs). Our micrometer-scale co-localization analysis in wounded zones suggests that linolenic acids are predominantly released from PCs with 16_18 fatty acid composition. A better understanding of plant molecular signaling pathways at a spatial and molecular level can aid in devising new approaches for the production of fuels and chemicals in bioenergy crops.

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