Title: Coupled Metabolomics and Transcriptomics Analyses Reveal Active Dynamics of Infection in Virocells

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Project Goals:

The overarching goal of this project is to establish ecological paradigms for how viruses alter soil microbiomes and nutrient cycles by developing foundational (eco)systems biology approaches. Within this overall project, we established bacterial virus (phage)-host model systems to investigate metabolic reprogramming in virus-infected cells (a.k.a. virocells). Such work is critical for establishing baseline approaches for understanding microcells across ecosystems, including soils, and to translate these findings into biogeochemical data needed to develop predictive computational models of soil microbial functioning and impacts.

Abstract:

Viruses control the microbes that provide essential planetary services through infection. Yet, studying them in complex ecosystems such as soils presents many challenges, ranging from establishing laboratory model systems to analyzing infections and measuring viral infection impacts on the ecosystem. To develop foundational approaches to study viruses in soils, here we used a known, ecologically-relevant bacterium (Pseudoalateromonas) and two contrastingly different infecting phages with (podovirus HP1 and siphovirus HS2) as model system for analytical tool development for analyzing infections and measuring their ecosystem impacts. Specifically, we chose a nutrient-challenged environment (phosphorus, P, limitation) to mimic natural conditions. Here we sought to push multi-omics approaches for two *Pseudoalteromonas* phage-host model systems (podovirus HP1 and siphovirus HS2) to improve mechanistic understanding of how phage and host respond to each other during infection with and without phosphorus (P) limitation. Because metabolite profiling responses are particularly under-studied in these systems, we also measured extra-cellular metabolites via high-resolution liquid chromatography-tandem mass spectrometry (LC-MS/MS) to follow the dynamics of database-captured exometabolites. This revealed a dynamic and complex response to phage infection in both virus-infected cells (a.k.a. virocells), with a notable increase in polyphenols in both virocells under P-limitation, though with contrasting temporal responses across the two virocells. While these molecules' production as a response to stresses is typical in plants, their production in bacterial cells due to phage infection has not yet been reported. To assess metabolomic response further, we leveraged MAGI (metabolite, annotation, and gene integration), which revealed fatty acid metabolites were elevated in virocells under P-limitation. To improve viral gene annotations (e.g., polyphenolic biosynthesis genes are not annotated), we applied gene-metabolite correlation networks and found that the majority of genes correlating with polyphenols were of unknown functions as opposed to other classes of compounds that were linked to genes with known potential functions. Finally, metabolite-metabolite correlation networks, described as "fingerprints" of metabolic systems, revealed a dynamic viral infection pattern in both virocells at each infection time point. These networks revealed the potential underlying enzymatic system and biomarkers of infection as

indicated by clusters of metabolites separated by time. Together, these findings bring new biological understanding of phage-host interactions and the impacts of nutrient limitation on their dynamics, but also simply provide a roadmap for such analyses to be conducted as virocells are increasingly explored across diverse ecosystems. Our study highlights novel insights in phage-host interactions and provided tools that can extend to new soil model phage-host systems.

References/Publications

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2. Howard-Varona, C., Lindback, M. M., Bastien, G. E., Solonenko, N., Zayed, A. A., Jang, H., ... & Duhaime, M. B. (2020). Phage-specific metabolic reprogramming of virocells. *The ISME journal*, *14*(4), 881-895.

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