Multi-Omics Identifies Metabolic Targets in *Histoplasma capsulatum*

Histoplasma capsulatum is a dimorphic fungal pathogen and the causal agent of histoplasmosis, a lung infection that can become severe and potentially fatal, especially in immunocompromised individuals. Recognized for its clinical significance, *Histoplasma* has been designated as a priority fungal pathogen by the World Health Organization. This human fungal pathogen is capable of surviving and replicating within macrophages, creating a significant challenge for host defense and therapeutic intervention. Once internalized into the nutrient-limited phagosome, the pathogen must extensively reprogram its metabolism to support intracellular growth.

To better understand the metabolic strategies that enable this adaptation, we applied a multi-omics approach combining metabolomics, transcriptomics, and ^{13}C-based metabolic flux analysis (aka fluxomics). This systems-level approach enabled the quantitative mapping of central carbon metabolism and the identification of metabolic adaptations that occur during infection. Mass spectrometry-based metabolomics was used to profile le levels of intracellular and extracellular metabolites under varying carbon sources, while transcriptomic data provided complementary insights into gene expression. To quantify *in vivo* reaction rates and carbon flow, we applied ^{13}C-stable isotope labeling and computational flux modeling, revealing key features of *Histoplasma*'s metabolism, such as the activity of the mannitol cycle and the methylcitrate cycle. It is important to note that these pathways are specific to *Histoplasma* and absent in mammalian cells, underscoring their potential as novel metabolic targets.

By combining these complementary techniques, our study identifies key regulatory nodes, alternative pathways, and metabolic bottlenecks essential for fungal survival and proliferation within host cells. This work provides the most detailed map to date of central carbon metabolism in pathogenic *Histoplasma* yeasts and establishes a foundational framework for dissecting infection-enabling metabolism. Ultimately, these insights support rational therapeutic design by revealing metabolic vulnerabilities that may serve as promising targets for new antifungal interventions.