

**RESEARCH**

Butkovich, L.V.; Leggieri, P.A.; Lillington, S.P.; Navaratna, T.A.; Swift, C.L.; Malinov, N.G.; Zalunardo, T.R.; Vining, O.B.; Lipzen, A.; Wang, M. **Separation of life stages within anaerobic fungi (Neocallimastigomycota) highlights differences in global transcription and metabolism.** *Fungal Genetics and Biology* 2024, 103958.

**Abstract:** Anaerobic gut fungi of the phylum Neocallimastigomycota are microbes proficient in valorizing low-cost but difficult-to-breakdown lignocellulosic plant biomass. Characterization of different fungal life stages and how they contribute to biomass breakdown are critical for biotechnological applications, yet we lack foundational knowledge about the transcriptional, metabolic, and enzyme secretion behavior of different life stages of anaerobic gut fungi: zoospores, germlings, immature thalli, and mature zoosporangia. A Miracloth-based technique was developed to enrich cell pellets with zoospores - the free-swimming, flagellated, young life stage of anaerobic gut fungi. By contrast, fungal mats contained relatively more vegetative, encysted, mature sporangia that form films. Global gene expression profiles were compared from two sample types (zoospore-enriched cell pellets vs. mature mats) harvested from the anaerobic gut fungal strain *Neocallimastix californiae* G1. Despite cultures being grown on glucose, the fungal zoospore-enriched samples were transcriptionally primed to encounter plant matter substrate, as evidenced by upregulation of catabolic carbohydrate-active enzymes and putative carbohydrate transporters. Furthermore, we report significant differential gene expression for gene annotation groups, including putative secondary metabolites and transcription factors. Understanding global gene expression differences between the fungal zoospore-enriched cells and mature fungi aid in characterizing fungal development, unmasking gene function, and guiding cultivation conditions and engineering targets to promote enzyme secretion.