## Resolving Intracellular Chloroplast in Fungi from Sequence to Slide.

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https://genomicscience.energy.gov/research/sfas/lanlbfi.shtml

Project Goals: This project intends to inform the use of soil microbiomes to address DOE priorities in overcoming energy and environmental challenges. We are focusing on understanding the role of bacterial:fungal interactions in ecosystem development by connecting microbial diversity to actionable phenotypic responses. To do so, genomic and metagenomic sequencing of soil microbes will be combined with advanced imaging techniques and metabolomics to determine a mechanistic route in which these organisms associate to augment soil fertility and plant growth.

**Abstract:** Surveying the metagenomes of soil microbiomes has led to the understanding that there is an intricate network of microbes that interact with each other both extra- and intracellularly. Fungi contribute largely to the complexity of soil ecosystems and 16S amplicon sequencing revealed that they house their own diverse microbiomes comprised of previously unreported bacterial endosymbionts. More impressively, 16S signatures of plant chloroplasts have also been observed and appear to persist within the fungi across generations. Here, we sought to investigate the existence of chloroplasts within fungi at the cellular and molecular level. Using amplified fluorescence *in situ* hybridization techniques we were able to visualize abundant signals closely corresponding to chloroplast spatially distributed across the fungal hyphae of several environmental isolates. Understanding how chloroplasts can be utilized and maintain within Fungi may greatly change our perspective to the role of chloroplasts across kingdoms

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of both the host fungus and these closely related endobacterial partners suggest this relationship could be valuable in establishing an experimental model for studying bacterial-fungal interactions.

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