m-CAFEs Applications of Targeted Editing in Microbial Networks

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Project Goals: Microbial Community Analysis and Functional Evaluation in Soils (mCAFEs) will use fabricated ecosystems (EcoFABs) in combination with CRISPR-Cas and phage-based approaches for interrogating gene and microbial functions *in situ* to gain critical new insights into the rhizosphere thus advancing a mechanistic understanding of microbial ecology. We will use 'bottom-up' defined microbial assemblies that enable detailed characterization of both constituent isolates and synthetic communities. This will be complemented by 'top-down' investigation of native soil-derived enriched microbial communities enabling extension of our approaches to more diverse communities that include uncultivated microbes. Predictive models will be developed and iteratively refined through integrated simulations and experimentation.

Through targeted editing, the m-CAFEs program seeks to elucidate the complex biological roles and functional genomics of individual members of microbial networks within the rhizosphere. Current methods for the targeted editing of mixed communities are bound by low delivery efficiencies, as well as the breadth of target specificities. To overcome these limitations, the m-CAFEs program is leveraging the characteristically high delivery efficiencies and narrow host range of bacteriophages to deliver DNA-targeting CRISPR-Cas endonucleases to select microbes of interest. Through the characterization of ablation efficiency and host specificity of various phages, as well as the isolation of novel phages, we have assembled a library of candidate phages for the manipulation of our synthetic community. We demonstrate the efficiency of phage-based ablation both *in vitro* and within EcoFAB devices and show that these phages can successfully target a host of interest without perturbing other members of the community. Further, sequencing candidate phages within our library, we have identified target loci for the engineering of CRISPR-Cas endonucleases, a critical step toward the implementation of targeted editing. Continued development of this platform will extend our capabilities beyond targeted ablation to enable the addition of desirable traits for functional enhancement of rhizosphere communities, which could be of particular significance to the evaluation of plant fitness in future models. Although these tools will have an immediate impact on the research of synthetic communities, the ultimate utility of these tools lies in its translational application to uncultivable microbial communities, facilitating the functional characterization of microbes that cannot be readily isolated.

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