

Microbial Community Assembly and Evolution Analyzed at Single Cell Level

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Project Goals: ENIGMA (Ecosystems and Networks Integrated with Genes and Molecular Assemblies) uses a systems biology approach to understand the interaction between microbial communities and the ecosystems that they inhabit. To link genetic, ecological, and environmental factors to the structure and function of microbial communities, ENIGMA integrates and develops laboratory, field, and computational methods. Understanding the general principles and mechanisms for community assembly, evolution and collapse across different environments and scales.

A two-organism model community was previously established under sulfate-deplete conditions that prevented independent growth of *Desulfovibrio vulgaris* (Dv) by sulfate respiration (SR), and required its syntrophic (ST) interaction with *Methanococcus maripaludis* (Mm) to support growth of both organisms. Experimental evolution of this model community in a uniform resource environment significantly improved growth characteristics across all 12 lines at the expense of erosion of SR. On the other hand, growth in a fluctuating resource environment that required the community to switch back and forth between SR and ST led to population collapse. We probed the evolution and collapse phenomena by performing bulk and single cell genomics and transcriptomics across various stages of evolution, and compared these data with similar analyses of phenotypically characterized simplified communities, and clonal isolates. This multiscale systems analyses revealed that during evolution of obligate ST in a uniform resource environment, many independent adaptive strategies had simultaneously co-evolved within the same evolution line. This finding was confirmed upon observing that distinct pairings of Dv and Mm obtained through end-point dilutions (EPDs) from the same evolution line produced higher growth rates and yields, relative to cross-pairings of the isolates across EPDs. Multiscale analysis of the community subjected to the fluctuating environment on the other hand revealed that the phenomenon of collapse had occurred due to excessive regulation of genes, which had driven progressive dilution of essential transcripts and proteins. Together, these studies showcase how multiscale systems analysis can reveal signatures, and timelines of adaptive molecular events and the mechanisms by which they shape the assembly, evolution and collapse of microbial communities.

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