

**Title: The role of adaptive evolution in shaping the structure and function of model microbial consortia**

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**Project Goals:** Laboratory based studies of nascent and evolved model microbial consortia are used to develop a predictive - rather than an observational – understanding of environmental processes. The general aim of these studies is to examine the role of adaptive evolution in determining community stability and productivity. Specific project aims include the identification of mutations correlated with improvements in community efficiency (as measured by conversion of substrates to microbial biomass) and community stability, and the determination of the contribution of individual mutations to community efficiency and stability. The project is part of a large research effort within ENIGMA consortium, entitled ‘Evolution and Assembly of Communities’, which aims to understand the impact of adaptive evolution on system-level processes.

**Abstract:** Syntrophy is a widespread relationship between microbes that plays a central role in the decomposition of carbon in anoxic environments. In these interactions, bacteria ferment organic acids to produce hydrogen, formate or acetate, which are consumed by hydrogeno- and aceto-trophic methanogens. Removal of the byproducts of the fermentation benefits bacteria because the free energy ( $\Delta G$ ) decreases with decreasing concentrations of the byproducts. A number of bacterial species seem to be specialized to ferment organic acids in syntrophic associations with hydrogenotrophic species. The question is how and why this specialization evolved? The question was addressed by examining the evolution of experimentally established obligate syntrophy between the sulfate reducing bacterium *Desulfovibrio vulgaris* Hildenborough (DvH) and the archaeon *Methanococcus maripaludis* (Mmp) (1). The cocultures evolution was followed over 1000 generations in two environments, one allowing for the heterogeneous distribution of resources and the other not. Evolved cocultures showed significant improvements in stability, growth yields and growth rates by the 300<sup>th</sup> generation, suggesting a capacity for rapid adaptive evolution of both partners (1). Genome resequencing of 1000<sup>th</sup> generation evolution lines revealed that the loss of a capacity for sulfate respiration, due to loss-of-function mutations in the *aps* and *sat* genes, was a common evolutionary outcome for 13 out of 21 replicate evolution lines (2). Genome resequencing also revealed the emergence and coexistence of distinct evolved strains of DvH and Mmp, all apparently contributing to

significant improvements in growth of the cocultures (2). These observations led to further questions such as: i) what combinations of mutations are responsible for the observed improvements? ii) at what stage of the evolution did these mutations arise? and iii) what are the benefits of these mutations to the individual and to the community?

To address the first two questions, we sequenced the genomes of 72 clonal isolates from three 1000 generation evolution lines and obtained metagenomes from the earlier time points in the evolution (prior to the 1000<sup>th</sup> generation). The analysis of mutations in clonal isolates showed that all strains were enriched in mutations in genes related to signal transduction, energy production/conversion, and nutrient transport. Interestingly, all 36 DvH clonal isolates represent unique genotypes and only few common mutations were found. Hypothetical porin (DVU0799), UTP-glucose-1-phosphate uridylyltransferase (*galU*, DVU1283) and long-chain fatty acid transporter protein (DVU1260) were among the few genes mutated in all clonal isolates. Physiological and biochemical characterization of these mutated genes suggested they contribute to improved inter-species cooperation by facilitating the direct transfer of nutrients/metabolites by promoting aggregation and increasing membrane permeability. Mutations in regulatory genes (transcript regulators and histidine kinases) associated with energy metabolism, lactate utilization and motility suggested that functional modification of these central processes also played important roles in adaptive evolution. For example, mutations in Mmp clonal isolates were concentrated in amino acid transport systems (MMP0166, MMP0419 and MMP1511) and in the regulator of archaellum (MMP1718).

Genotypic information is now being complemented by detailed phenotypic characterization of earlier time points in evolution and of selected clonal isolates grown in mono- and co- culture. Preliminary analysis of metagenomes from the earlier times of evolution suggests an early emergence of common mutations, followed by rapid and repeated gain and loss of mutations as these simple communities evolved to become more stable and productive. Improvement in co-culture growth was significant even if the pairings were from clones isolated from different evolution lines. Notably, growth of evolved DvH clonal isolates in monoculture also showed significant improvements in yield when grown in the same medium, suggesting that one common adaptive feature of adaptive evolution involved changes in maintenance energy requirements.

## References

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