ENIGMA Laboratory: Evolutionary and Ecological Origins of Community Assembly, Stability, and Efficiency

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Project Goals: A goal of DOE and ENIGMA is to understand and ultimately predict microbial community assembly and the adaptive response of communities to environmental change. To address these questions, we are examining assemblies of simple two-member communities composed of a secondary fermenter like *Desulfovibrio* species and hydrogenotrophic methanogens. This type of association (syntrophy) is representative of a trophic interaction sustaining both pollutant transformation and organic matter mineralization in many anoxic environments typical of the subsurface. To study the metabolic versatility and specificity of these assemblies, we first investigated the association of twelve different pairings of *Dv.* species and two methanogenic species (*Methanococcus maripaludis* and *Methanospirillum hungatei*). The results demonstrated that different *Desulfovibrio*-methanogen pairings vary significantly in their growth characteristics, most notably in their ability to ferment lactate at elevated hydrogen levels, presumably reflecting differences in their syntrophy-associated enzyme systems (e.g., hydrogenases and electron transfer complexes). Those studies now serve to direct a systems-level approach to the study of common and divergent features of community interaction: focusing on the genetic and metabolic signatures of efficient species interaction, major determinants of community stability, and the capacity for these communities to improve through adaptive evolution.

Abstract: Comparative studies have so far shown that both the electron transfer system and the mediator for electron transfer differ among *Desulfovibrio* species. For example, comparison of different assemblies grown in chemostats under various dilution rates demonstrated the importance of formate as a major mediator of electron exchange in *Dv. alaskensis* strain G20 syntrophic cocultures in contrast to the hydrogen exchange-based system of *Dv. vulgaris* strain Hildenborough. Notably, the transcript analyses revealed that gene expression during syntrophic growth of *Dv. alaskensis* str. G20 also varies with both growth rate and the methanogenic partner. These conclusions were subsequently confirmed using a tagged-transposon *Dv. alaskenis* G20 mutant mini-library (1200 strains) to examine the relative fitness of different insertion mutants grown syntrophically in chemostats. Complementary studies are examining the adaptive evolutionary response of the two species to syntrophic growth. Ongoing laboratory evolution experiments of 24 replicated lines have so far documented a remarkable capacity for rapid improvement in the stability and efficiency of this mutualism after only 1000 generations of cooperative growth. The genetic basis of improved cooperation is now being examined by genome resequencing, initially of twelve of the pairings at 1000 generations using both Illumina and SOLiD next generation sequencing platforms and microarrays. Since some lines have evolved to obligate syntrophy, the history of their adaptive evolution will be reconstructed using single cell genome sequencing of earlier generations. The first mutations in *Dv. vulgaris* to become fixed in multiple lines were in an outer membrane porin (DVU0799), suggesting that amino acid replacements near the outer face of this porin alters the flux of metabolites and/or

nutrients. Other notable and frequent mutations were in genes implicated in EPS synthesis and regulation of lactate metabolism. Collaborative biophysical studies with ENIGMA members are now exploring altered function of the different porin mutants and mutants implicated in EPS synthesis. Of particular note was the replacement in all evolved lines of six amino acids in the sensory PAS domain of a histidine kinase (DVU3022) implicated in the regulation of lactate metabolism. The high frequency replacement with an identical short amino acid sequence having predicted sensory function suggests a novel phase variation-like mechanism of adaptive response. Together these ongoing studies point to both common and divergent mechanisms of interspecies interaction, and offer a framework to better resolve genetic, structural, and metabolic features contributing to stability and efficiency of community assembly.

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