85. Enhancing H_2-Production and Nutrient Exchange in a Symbiotic Bacterial Coculture

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Project goals: Specialized features of individual microbial species can be combined in a complementary manner in cocultures or consortia to produce useful fuels and chemicals. The challenge of maintaining stable microbial relationships has impeded progress in characterizing and implementing such consortia. The goals of our project are to develop a stable hydrogen gas-producing microbial coculture and to use genetic, biochemical, evolutionary, and systems biology approaches to characterize and manipulate microbial interactions and enhance hydrogen production.

Hydrogen gas is an important commodity chemical and is being considered as a future biofuel. Many fermentative bacteria, like *Escherichia coli*, produce H_2 from carbohydrates under anaerobic conditions but at low yields due to the obligate excretion of organic acids and alcohols. Photosynthetic purple nonsulfur bacteria, like *Rhodopseudomonas palustris*, consume fermentation products under anaerobic conditions and use some of the electrons to produce H_2 via nitrogenase. It has long been realized that combining these two classes of microbes can result in higher H_2 yields from carbohydrates (1). However, little progress has been made with such cocultures in the last thirty years likely due to the non-trivial challenge of maintaining stable relationships between the two species. Using defined mutations and environmental conditions we recently developed a stable coculture of *E. coli* and *R. palustris*. In this coculture *E. coli* ferments carbohydrates and excretes carbon for *R. palustris* and *R. palustris* fixes N_2 gas and excretes nitrogen for *E. coli*. One species cannot survive without the other.

We are currently examining the environmental, metabolic, and evolutionary factors that influence coculture H_2 production and nutrient exchange. An environmental perturbation that profoundly affects the H_2 yield is whether the cocultures are shaken or not. When cocultures are not shaken, N_2 gas diffusion is limited and results in a subpopulation of *R. palustris* that is starved for nitrogen. When starved, *R. palustris* still metabolizes carbon and redirect electrons away from biosynthesis to H_2 production (2). The H_2 yield from non-shaken cocultures was 17- times higher than in shaken cocultures. Despite the starving subpopulation, non-shaken cocultures were still viable with reproducible trends through serial transfers.

A metabolic factor that affects coculture H_2 production and nutrient exchange is the CO_2-fixing Calvin cycle. The Calvin is known to compete for electrons against H_2-production via nitrogenase in *R. palustris* monocultures and genetically disrupting Calvin cycle flux leads to higher monoculture H_2 yields (3). We recently determined that deleting the genes encoding the Calvin cycle enzyme, Rubisco, results in a lower *R. palustris* monoculture growth rate than when the gene encoding the Calvin cycle enzyme, phosphoribulokinase, is deleted (4). The low Rubisco mutant growth rate is likely the result of toxic metabolite accumulation that is avoided in phosphoribulokinase mutants (5). Furthermore, we found that phosphoribulokinase mutants have a higher specific rate of H_2 production than Rubisco mutants in monoculture. In coculture, we similarly found that genetically disrupting *R. palustris* Rubisco activity led to a lower coculture growth rate. However, disrupting *R. palustris* phosphoribulokinase activity led to a higher coculture growth rate, likely due to an increased redirection of electrons to nitrogenase and higher rate of nitrogen excretion. Cocultures with either Calvin cycle mutant produced more H_2 than cocultures containing the *R. palustris* parent strain.

We will continue to use our ability to genetically manipulate each species as we determine the effect of *E. coli* fermentation product profiles on coculture traits and ultimately employ transcriptomic and fluxomic approaches to characterize interspecies interactions in this simple defined community.

References:

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