

Factors Governing Mutualism Dynamics in a Hydrogen-Producing Coculture

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Project Goals: The goals of this project are to (i) develop a stable hydrogen gas-producing coculture between *Rhodopseudomonas palustris* and fermentative microbes, such as *Escherichia coli*, (ii) use genetic, biochemical, evolutionary, and systems biology approaches to characterize and manipulate microbial interactions and H₂ production.

Synthetic microbial communities, or cocultures, preserve natural interactions while allowing for an expanded range of experimental approaches and control. Cocultures are thus valuable for addressing ecological and evolutionary questions, such as how microbial communities transform carbon. Cocultures can also combine diverse traits to convert renewable resources into fuels and other useful chemicals. However, establishing cocultures that support stable coexistence and yield reproducible results is often challenging.

We developed an anaerobic coculture between fermentative *Escherichia coli* and an engineered strain of phototrophic *Rhodopseudomonas palustris* (Nx) that together convert carbohydrates into H₂ gas, a potential biofuel. The two bacteria form a syntrophic relationship wherein *E. coli* ferments glucose and excretes essential carbon (organic acids) for *R. palustris* while *R. palustris* fixes N₂ and excretes essential nitrogen (NH₄⁺) for *E. coli*. The bidirectional exchange ensures stable coexistence as two populations converge on a common equilibrium from starting ratios spanning over six orders of magnitude favoring either species. Growth and metabolic trends are highly reproducible over serial transfers. We have also developed kinetic models that accurately describe coculture dynamics and can be used to predict the effects of various perturbations^{1,2}. We are now using computational and experimental approaches to address the importance of core metabolic traits in deciding the phenotypic behavior of this community.

To generate ATP, *E. coli* must excrete organic acids under fermentative conditions. Thus, even under complete nitrogen-starvation, *E. coli* will continue to ferment glucose to organic acids to maintain itself. This trait has important implications for coexistence². Our model suggested that growth-independent fermentation is critical for sustaining coexistence at low levels of NH₄⁺ transfer. When growth-independent fermentation was excluded from the model, the coculture was predicted to go extinct below a threshold level of NH₄⁺ transfer. We decreased NH₄⁺ transfer in our coculture by lowering the supply of N₂. Coexistence was observed at even the lowest levels of NH₄⁺ transfer that we tested, suggesting an essential role for growth independent fermentation. The continuous excretion of organic acids stimulates *R. palustris* growth and reciprocal NH₄⁺ excretion, eventually lifting both species out of starvation. Organic acids are an obligate waste product of fermentative organisms and an important carbon and electron shuttle in anaerobic communities. Thus the importance of growth-independent fermentation in establishing and maintaining cross-feeding relationships could be widespread in nutrient-limited environments. The highest coculture H₂ yields were also observed under severely nitrogen-limiting conditions, exceeding the theoretical maximum fermentative H₂ yield.

We also found that growth-independent fermentation can be detrimental to coexistence when the *E. coli* population is large. Such large populations amplify the rate of growth independent fermentation and result in rapid organic acid accumulation that acidifies the medium before growth can occur. This result highlights a dual role for organic acids, moving along a continuum between being beneficial versus detrimental.

We also determined that the relative benefit *R. palustris* receives from organic acids can be influenced by its own level of cooperative NH_4^+ excretion¹. Our model predicted that more NH_4^+ excretion would result in a less efficient utilization of feedstock and a lower *R. palustris* cell density. The higher levels of NH_4^+ would stimulate rapid *E. coli* growth and organic acids would be produced faster than *R. palustris* could consume them, resulting in a growth-inhibiting acidic pH. Thus, by cooperating more, *R. palustris* would change the nature of organic acids from a carbon source to a growth inhibitor. To test these predictions, we engineered a ‘hyper-cooperator’ strain of *R. palustris* that excretes 3-fold more NH_4^+ than the Nx parent. Cocultures with the hyper-cooperator confirmed the predictions, as there were fewer *R. palustris* cells, a higher residual organic acid concentration, and a more acidic pH. Nonetheless, the hyper-cooperator and *E. coli* stably coexisted over serial transfers, albeit at a new equilibrium.

Our results inform on the potential for both positive and negative roles of organic acids in anaerobic food webs and the influence that microbial interactions can have on deciding these roles. Our results also have implications for the use of engineered NH_4^+ -excreting, N_2 -fixing bacteria in both industrial and agricultural settings. Varied NH_4^+ excretion rates can indirectly modify system behavior in potentially negative ways, such as altering the species ratio or misdirecting resources towards undesired products. In other words, optimizing NH_4^+ cross-feeding does necessarily mean increasing NH_4^+ cross-feeding.

References

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