Enhanced Cross-Feeding in a Bacterial Coculture Undermines a Mutualistic Relationship

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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 H_2 production.

Synthetic microbial communities, or cocultures, preserve core aspects of microbial interactions found in natural environments while allowing for an expanded range of experimental approaches and control. Cocultures are thus valuable for addressing questions in microbial ecology and evolution, such as how microbes transform carbon as a community. Cocultures can also combine complementary traits from diverse microbes to convert renewable resources into fuels and other useful chemicals. However, establishing cocultures that support stable coexistence and yield reproducible results has proven challenging in many cases.

Our work focuses on an anaerobic coculture between fermentative *Escherichia coli* and an engineered strain of phototrophic *Rhodopseudomonas palustris* (Nx) that together convert carbohydrates into H_2 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* Nx while *R. palustris* Nx fixes N_2 and excretes essential nitrogen (NH_4^+) for *E. coli*. The bidirectional exchange ensures stable coexistence as the two populations grew to a common equilibrium from starting ratios spanning over twelve orders of magnitude. Growth and metabolic trends were highly reproducible over serial transfers, motivating us to develop an ecological model that accurately describes these trends.

Exchange of metabolites is an important basis for many mutualistic systems. However, the impact of metabolite exchange rates on the bacteria involved is typically difficult to address. Using our model, we simulated the effects of higher *R. palustris* NH_4^+ excretion rates. The model predicted that more NH_4^+ excretion would result in a less efficient utilization of feedstock and a lower final *R. palustris* cell density. Essentially, higher levels of NH_4^+ would stimulate faster *E. coli* growth and organic acids would be produced faster than *R. palustris* could consume them, eventually resulting in a growth-inhibiting acidic pH. 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 generated 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.

Our results have implications for the deployment of engineered NH_4^+-excreting diazotrophs as biofertilizers for agricultural and industrial applications and perhaps for other engineered cross-feeding systems. 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 metabolite excretion rates for cross-feeding does necessarily mean increasing the excretion rates.

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