Competition for a cross-fed nutrient between bacterial mutualists

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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 the factors governing 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 in favor of either species1. 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 perturbations1-3. We are now using computational and experimental approaches to address the importance of core metabolic traits in deciding the phenotypic behavior of this community, which resembles organic acid cross-feeding interactions found in anaerobic food webs and nitrogen cross-feeding found in various environments.

Previously we determined that the level of organic acid excretion by *E. coli*, which is directly influenced by the level of NH4+ excretion by *R. palustris*, determines the relative benefit that *R. palustris* receives from organic acids1,2. At high levels of excretion, organic acids become detrimental rather than beneficial as they acidify the environment and inhibit growth1. At low levels of excretion, organic acids can sustain the mutualism even through periods of starvation2. More recently we have focused on the role NH4+, which unlike organic acids, is not a waste
product of metabolism but rather a metabolite that both species value as a nutrient. We thus explored how interpartner competition for a communally valuable cross-fed nutrient impacts mutualism dynamics. By decreasing the affinity of each species for NH$_4^+$ both computationally and by genetically disrupting NH$_4^+$ transporters, we discovered that mutualism stability necessitates that the recipient, 	extit{E. coli}, have a competitive advantage against the producer in obtaining the cross-fed nutrient, provided that the nutrient is generated intracellularly$^3$. RNAseq and proteomics analysis, along with genetic disruption of the 	extit{E. coli} master regulator of nitrogen metabolism, NtrC, revealed that the nitrogen starvation response is crucial for 	extit{E. coli} to assume a physiological state that reliably maintains coexistence with 	extit{R. palustris}. We propose that the requirement for recipient-biased competition is a general rule for mutualistic coexistence based on the transfer of intracellularly generated, communally valuable resources.

**References**
3. McCully, AL, B LaSarre, and JB McKinlay. 2017. Recipient-biased competition for an intracellularly generated cross-fed resources is required for coexistence in a bacterial mutualism. mBio. 8: e01620-17

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