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 H_2 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 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* while *R. palustris* fixes N_2 and excretes essential nitrogen (NH_4^+) 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 species[1]. 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[1-3]. 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 NH_4^+excretion by *R. palustris,* determines the relative benefit that *R. palustris* receives from organic acids[1,2]. At high levels of excretion, organic acids become detrimental rather than beneficial as they acidify the environment and inhibit growth[1]. At low levels of excretion, organic acids can sustain the mutualism even through periods of starvation[2]. More recently we have focused on the role NH_4^+, 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, *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 *E. coli* master regulator of nitrogen metabolism, NtrC, revealed that the nitrogen starvation response is crucial for *E. coli* to assume a physiological state that reliably maintains coexistence with *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

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