163. Characterization of an Obligately Syntrophic H2-producing Bacterial 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 Escherichia coli, (ii) use genetic, biochemical, evolutionary, and systems biology approaches to characterize and manipulate microbial interactions and H2 production, and (iii) establish stable cocultures between R. palustris and other fermentative microbes.

Synthetic microbial communities can be a valuable experimental system to understand microbial interactions and coevolution. Synthetic communities also offer opportunities to combine complementary metabolic traits to convert renewable resources into fuels and other useful chemicals. However, the utility of such systems often hinges on the ability on maintain a stable productive relationship between the species in the synthetic community.

Our work focuses on a synthetic anaerobic community composed of fermentative Escherichia coli and photoheterotrophic Rhodopseudomonas palustris. The coculture converts carbohydrates into H2 gas. E. coli produces H2 gas from carbohydrates but at a low yield due to the obligate production of organic acids and alcohols. R. palustris consumes fermentation products and use some of the electrons to produce H2 gas via nitrogenase. It has long been realized that combining these two lifestyles results in higher H2 yields from carbohydrates (1). However, progress has been impeded by the challenge of maintaining stable relationships. Through defined mutations and environmental conditions we developed a stable coculture of E. coli and R. palustris. As in previous cocultures, E. coli ferments carbohydrates and excretes essential carbon for R. palustris. Our system is stabilized by requiring that R. palustris fix N2 gas and excrete essential nitrogen for E. coli. One species cannot survive without the other.

We are examining the environmental, metabolic, and evolutionary factors that influence coculture productivity and nutrient exchange. Use of N2 versus NH4+ as the sole nitrogen source has profound effects on the species ratio, H2 productivity, and coculture stability. Depending on whether the cocultures are shaken or left static also has profound effects on the H2 yield. Static cocultures are expected to limit N2 diffusion into the medium and thereby induce N2 starvation. However, even under static conditions, cocultures remain viable and give reproducible results through serial transfers.

We have also begun to explore whether other industrially-relevant fermentative microbes can be substituted for E. coli in the coculture. When we attempted to coculture R. palustris with the ethanol-producing bacterium, Zymomonas mobilis, our negative controls lacking R. palustris also grew. This led to the discovery that Z. mobilis has the native ability to fix N2 gas (2). Remarkably, fixing N2 did not detract from the ethanol yield. Rather, the ethanol yield remained near the theoretical maximum during growth with N2. Growth with N2 also resulted in a higher specific rate of ethanol production and less residual biomass, compared with growth with ammonium.

N2-fixing Z. mobilis is potentially well-suited for cellulosic ethanol production as it does not require traditional nitrogen supplements needed to make up for the low-nitrogen contents of cellulosic feedstocks.

References:

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