

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 *Rhodospseudomonas 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 to 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 *Rhodospseudomonas 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 (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:

1. Odom JM, Wall JD. 1983. Photoproduction of H₂ from cellulose by an anaerobic bacterial coculture. *Appl Environ Microbiol* 45:1300–1305.

2. Kremer TA, LaSarre B, Posto AL, McKinlay JB. 2015. N₂ gas is an effective fertilizer for bioethanol production by *Zymomonas mobilis*. Proc Natl Acad Sci USA doi: 10.1073/pnas.1420663112.

This work was supported by the U.S. Department of Energy, Office of Science, Office of Biological and Environmental Research, under award number DE-SC0008131.