46. Understanding the carbon and hydrogen flow in constructed H_2-producing co-cultures

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Project goals: Microbial communities have multiple pathways for energy conversion and nutrient transfer. Which pathways dominate and how these pathways are interconnected greatly affects community function. Microbiologists have also started to appreciate the fact that the whole may be much more than the simple sum of its parts. The interactions between different components of a system result in many new physiological functions that cannot be observed with individual components. Our goal here is to develop genome-level models of carbon and energy flow within simplified microbial communities (co-cultures), with H_2 production as a metric for monitoring the overall state of the system.

In order to develop a defined ecosystem and to study the community metabolic interactions in detail, we have isolated and characterized a broad suite of microbial mat community members from the intertidal mats of Elkhorn Slough, CA. We have successfully isolated (as true isolates or stable consortia) all the major functional groups of the natural intertidal mat ecosystem including sulfur-oxidizing and -reducing bacteria, oxygenic and anoxygenic phototrophs, respiring and fermentative heterotrophs, and nitrogen-fixing and denitrifying bacteria, as well as the vast majority of the 15 most abundant taxa (as determined by metagenomics analysis of the mat community). With isolation efforts largely finished, we are now shifting our effort to examine interactions in mixed cultures containing 2 or more different functional groups. Preliminary successes include the construction of a stable light-driven sulfur-cycle between phototrophic purple bacteria and a sulfate-reducing bacterium; and the construction of cyanobacterial/fermentative co-cultures that considerably stimulate net H2-production. Our objective is to gain an emergent vision of what physiology and interactions facilitate stable and successful consortia in general ecological terms (*i.e.,* higher overall fitness, broader range of optimal growth conditions or high stability) as well as for practical applications (*i.e.,* high net H_2 productivity).

In parallel with constructing simplified microbial communities from natural systems, we have constructed an artificial co-culture containing *Clostridium cellulolyticum* H10 (CC) and purple bacterium *Rhodopseudomonas palustris* CGA676 (RP) for H_2 production based on cellulose degradation. Cellulose is the sole carbon and energy source for CC. RP utilizes fermentation products secreted by CC, and H_2 is produced by both organisms. To understand metabolism in this syntrophic system, we examined and compared the kinetics of cellular growth, H_2 production, and metabolite production/consumption in both mono- and co-cultures of these two taxa. Because both organisms have been fully sequenced, their co-culture is an ideal way to begin to understand the genomic underpinnings of syntrophy. Our results show that the presence of RP in co-culture greatly stimulates cellulose degradation and H_2 production, which likely results from accelerated metabolism of CC and consumption of acetate and pyruvate by RP. Using a high density microarray, we also investigated changes in CC's gene expression when co- cultured with RP. 291 genes had significantly different gene expression in co-culture, with 179 genes up-regulated and 112 down-regulated. Changes

in expression were calculated as an intensity ratio of CC co-culture versus CC monoculture. Many of the up- regulated genes are involved in translation, replication and cellulose breakdown, consistent with our observation of higher growth rates of CC when in co-culture. We also observed a significant increase in expression of a pyruvate kinase (16 fold) and a lactate dehydrogenase (8 fold), suggesting that CC produces more pyruvate and lactate when in co-culture.

In order to study system-wide carbon and energy flow within this co-culture, we are developing a genome-scale reconstruction of metabolism in CC and RP and are using Flux Balance Analysis (FBA) to study the metabolic capabilities of these organisms under different genetic and environmental conditions. To date, we have developed a system-level model of metabolism in RP and have used this model to examine the modes of metabolism most conducive to H_2 production. The model's predicted behaviors have been compared with experimental observations to ensure accuracy. As expected, our *in silico* analysis showed the RP does not require light to grow in nutritionally rich environments. Furthermore, we tested its ability to grow on organic acid byproducts of CC metabolism (acetate, ethanol, lactate and pyruvate). Consistent with results of fluxomic analysis, our results indicate that H_2 production is closely linked to CO_2 production. Carbon fixation results in reduced production of H_2. Consumption of carbon sources like ethanol and acetate that are more reduced than the cellular biomass results in greater production of H_2 in comparison to more oxidized compounds like pyruvate. Additionally, our *in silico* analyses indicate that RP has a very robust mechanism for autotrophic growth and is not dependent on the Calvin cycle for photoheterotrophic growth. This result has not been experimentally verified and is in disagreement with observed essentiality of Rubisco for photoheterotrophic growth in other purple- non- sulfur bacteria. Overall, since H_2 production is inversely linked to carbon fixation/conservation, increased H_2 yield adversely effects cellular growth.

Through the systems biology studies of these simplified co-cultures for hydrogen production, we have gained insights into key metabolic interactions and population dynamics in such co-cultures that are critical for improvement of syntrophic efficiency and H_2 productivity. Continued improvements in our ability to track metabolite fluxes will provide a more comprehensive picture of intermediary metabolism and the factors that control the flux of organic matter that result in H_2 production.

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