Phototrophic metabolism of organic compounds generates excess reducing power that can be redirected to produce H_2 as a biofuel

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Objectives

The objectives of this project are (i) to use ^{13}C-metabolic flux analysis and other approaches to identify metabolic factors that influence the phototrophic production of H_2 from organic compounds by *Rhodopseudomonas palustris* and (ii) to use the resulting information to guide the engineering of *R. palustris* for improved H_2 production.

Technical Barriers

A technical barrier to engineering bacterial strains for enhanced H_2 production is a limited ability to follow electron flow through metabolism. Here we used ^{13}C-metabolic flux analysis, in combination with physiological and other 'omics' analyses, to overcome this barrier by tracking fluxes through redox reactions.

Abstract

Although most manufactured H_2 comes from fossil fuels, H_2 can also be produced biologically. The bacterium *Rhodopseudomonas palustris* uses energy from sunlight and electrons from organic waste to produce H_2 via nitrogenase. In order to understand and improve this process we used ^{13}C-substrates having various oxidation states to track and compare central metabolic fluxes in non-H_2 producing wild-type *R. palustris* and an H_2-producing mutant. The pathways by which substrates were oxidized generated excessive amounts of reducing power such that only 40-60% could be used for biosynthesis, depending on the growth substrate. Wild-type cells relied heavily on the CO_2-fixing Calvin cycle to oxidize the excess reduced electron carriers, using CO_2 produced from the organic substrates by other metabolic reactions. The H_2-producing mutant used a combination of CO_2 fixation and H_2 production to oxidize excess reduced electron carriers. The majority of electrons for H_2 production were diverted away from CO_2 fixation for all substrates. Microarray and qRT-PCR analyses indicated that this shift of electrons towards H_2 involved transcriptional control of Calvin cycle gene expression. These observations pointed to the Calvin cycle as a convenient single target to disrupt to force more electrons towards H_2 production. Blocking Calvin cycle flux by mutation in the H_2-producing strain resulted in higher H_2 yields for all substrates. The increase in H_2 yield was proportional to the Calvin cycle flux in the parent strain for most substrates. These results demonstrate how systems level approaches, such as ^{13}C-metabolic flux analysis, can lead to effective strategies to improve product yield. Furthermore, our results underscore that the Calvin cycle and nitrogenase have important electron-accepting roles separate from their better known roles in biomass generation and ammonia production.

Progress Report

We established ^{13}C-labeling techniques to determine metabolic fluxes in batch cultures of *R. palustris*. This information provided key insights into the physiology of *R. palustris*, some of which provide a better understanding of its genome sequence (e.g., an additional isoleucine pathway was identified by the labeling studies that was missed during genome annotation), or could not have been predicted from the genome sequence (e.g., 80-90% of the NADPH needed for biosynthesis comes from a transhydrogenase-like reaction and not from canonical sources such as the pentose phosphate pathway and isocitrate dehydrogenase). Our data also quantitatively answered a long-standing question of why an electron accepting process (e.g., CO_2 fixation or H_2 production) is needed during photoheterotrophic growth on relatively oxidized organic compounds. Additionally, we identified the Calvin cycle as the primary competing pathway for electrons against H_2 production during growth on multiple substrates. We show that the Calvin cycle is a convenient, non-essential, single target for disruption that leads to higher H_2 yields during growth on all substrates tested.

Future Directions

Even in our Calvin cycle mutants, the majority of substrate electrons are incorporated into cellular biomass. For example, 15% of the electrons in acetate are used for H_2 production while 85% are used for biosynthesis. Thus, using non-growing cells should result in much higher H_2 yields. Indeed, *R. palustris* produces H_2 from acetate at 60% of the theoretical maximum yield when completely starved of nitrogen. *R. palustris* is potentially well suited for use as a non-growing biocatalyst since it can remain metabolically active for months in a non-growing state using light energy and cyclic photophosphorylation to satisfy maintenance

energy demands. By the same token, *R. palustris* can serve as a model bacterium for studying the physiology of non-growing cells. We are now determining the fate consumed electrons in nitrogen-starved *R. palustris* by quantifying excreted products and changes to its biomass composition. Additionally, we are adapting our ^{13}C-labeling protocols to determine metabolic flux distributions in non-growing *R. palustris*. The resulting information will be used to engineer *R. palustris* to improve the H_2 yield under non-growing conditions.

Publication list (including patents) acknowledging the DOE grant or contract

1. McKinlay, JB and CS Harwood. 2011. Calvin cycle flux, pathway constraints and substrate redox state together determine the H_2 biofuel yield in photoheterotrophic bacteria. *mBio.* In press.

2. McKinlay, JB and CS Harwood. 2010. Carbon dioxide fixation as a central redox cofactor recycling mechanism in bacteria. *Proceedings of the National Academy of Sciences USA*. 107: 11669-11675.

3. McKinlay, JB and CS Harwood. 2010. Mini-review. Photobiological production of hydrogen gas as a biofuel. *Current Opinion in Biotechnology.* 21: 244-251.