Using genomic investigations of the photosynthetic bacterium *Rhodobacter sphaeroides* to determine the origin of the increased H_2 production capacity of a mutant strain

Wayne S. Kontur[1,3,*] (wskontur@wisc.edu), Wendy Shackwitz[4], Joel Martin[4], Shweta Deshpande[4], Hope Tice[4], Kurt LaButti[4], Natalia Ivanova[4], **Daniel R. Noguera[2,3]** (noguera@engr.wisc.edu), and **Timothy J. Donohue[1,3]** (tdonohue@bact.wisc.edu)

[1]Department of Bacteriology and [2]Department of Civil and Environmental Engineering, University of Wisconsin-Madison; [3]DOE Great Lakes Bioenergy Research Center, Madison, WI; and [4]DOE Joint Genome Institute, Walnut Creek, CA

Project Goals

Our main goal is to use the purple nonsulfur photosynthetic bacterium *Rhodobacter sphaeroides* as a model for studying cellular electron flow in photosynthetic bacteria. Building off of our understanding of the distribution of electrons into various cellular pathways, we ultimately aim to develop *R. sphaeroides* and other photosynthetic bacteria into platforms for producing biofuels, including hydrogen gas (H_2). Here, we use microbiological and genomic analyses to investigate and characterize a high H_2-producing derivative (strain Ga) of wild-type strain *R. sphaeroides* 2.4.1.

Abstract

Rhodobacter sphaeroides is a metabolically diverse purple nonsulfur photosynthetic bacterium. During anaerobic photoheterotrophic growth (with light as an energy source and an organic substrate as a carbon and electron source), *R. sphaeroides* can produce hydrogen gas (H_2) via its nitrogenase enzyme. The wild-type strain 2.4.1 is capable of channeling up to 40% of the electrons it consumes from organic substrates into H_2, depending on the organic substrate.

We seek to identify cellular pathways that compete with H_2 production for electrons, either by recycling 'excess' electrons that are not utilized in primary biosynthetic pathways, or in the production of cellular components that are not necessary to the cell in an industrial H_2 production setting. Examples of these types of cellular pathways are CO_2 fixation via the Calvin Cycle, which the cell uses to recycle electrons even in the presence of a fixed carbon source, and the production of polyhydroxybutyrate (PHB), a carbon and electron storage polymer. Mutants in which these pathways have been inactivated show increased H_2 production capacities.

We find that strain Ga, an uncharacterized mutagenized derivative of wild-type 2.4.1, channels electrons into H_2 more efficiently than 2.4.1 (~56% of the electrons from succinate are converted into H_2 by Ga, versus ~35% of the electrons from succinate for 2.4.1). Ga grows more slowly and reaches lower final cell densities than 2.4.1, which suggests that some of the additional electrons channeled into H_2 by Ga may be coming at the expense of cellular biomass production.

In collaboration with the DOE's Joint Genome Institute, we have sequenced the genome of strain Ga. We find that 18 genes contain an insertion or deletion resulting in a frame shift between 2.4.1 and Ga. There are 56 additional genes that contain a single nucleotide polymorphism between 2.4.1 and Ga. We also find that a large (>38 kb) region, containing many viral associated genes, is missing from the Ga genome.

A comparison of the 2.4.1 and Ga genomic sequences has helped to identify the genomic origins of other differences between 2.4.1 and Ga not related to H_2 production. For example, mutations that result in inactive forms of methoxyneurosporene dehydrogenase (CrtD, an enzyme involved in carotenoid biosynthesis) and of fructokinase help explain, respectively, differences in pigmentation between the strains, and the deficiency of Ga in utilizing fructose as an organic substrate. The other genetic differences between the strains are being investigated to determine the origin(s) of Ga's heightened H_2 production capacity.