An Engineered Thermophilic Microbe Achieves High Ethanol Yields by Hydrogen Cycling via a Novel Redox System

Background/Objective

- *Thermoanaerobactium thermosaccharolyticum* is a hemicellulose- and sugar-consuming bacterium that has been genetically engineered for ethanol production yields >85%, and is capable of generating ethanol titers >50 g/L.
- However, the underlying reactions responsible for electron flow, redox equilibrium, and how they relate to ethanol production in this microbe are not fully understood.

Approach

- We performed a series of genetic manipulations to investigate the contribution of hydrogenase genes to hydrogen cycling to high ethanol yield, generating evidence for the importance of hydrogen-reacting enzymes in ethanol production.

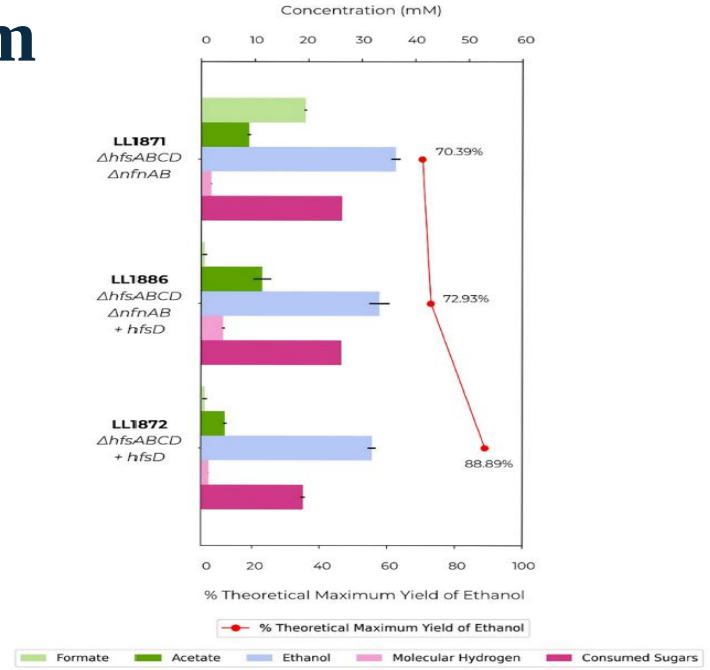
Results

- High ethanol yield in this organism only occurs when the *hfsD*, *hydAB*, and *nfnAB* genes are all present, and the *hfsB* gene is absent.
- We propose that the products of this three-gene cluster facilitate an NADPH-generating reaction via a novel mechanism of hydrogen cycling, allowing efficient redox control and increased ethanol yields.

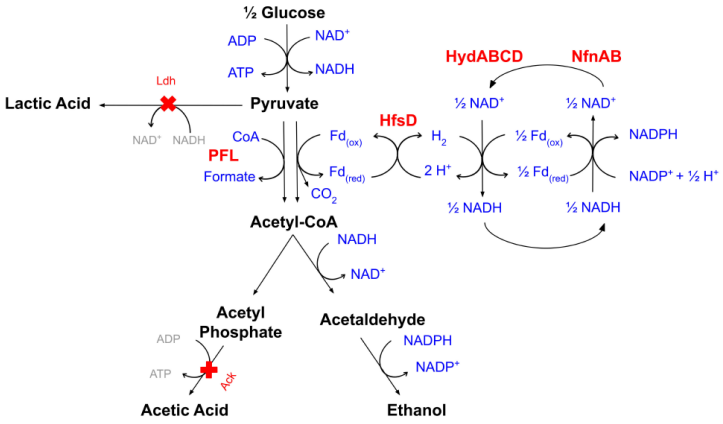
Significance/Impacts

- This work advances our understanding of co-factor balance and recycling in high-yield 'ethanologen' strains, providing key insights into optimizing energy conversion from biomass-derived sugars to ethanol. We will use these insights to improve ethanol yield and titer in other anaerobic thermophiles, such as the cellulose-utilizing *Clostridium thermocellum*. These findings contribute to improving fermentation efficiency and sustainable biofuel production.

deSouza, LC et al., *App. Environ. Microbiol.* (2025) e0110924. doi: 10.1128/aem.01109-24



***hfsD* and *nfnAB* are required for high ethanol yield**



Proposed mechanism for ferredoxin re-oxidation during ethanol production in *T. thermosaccharolyticum*