The Impact of AdhE Mutations on Ethanol Production in *T. saccharolyticum*

Background/Objective

- *Thermoanaerobacterium saccharolyticum* is a thermophilic bacterium engineered for high-yield ethanol production. Bifunctional alcohol dehydrogenase E (AdhE) is an enzyme in *T. saccharolyticum* that is central to microbial fermentation and a frequent target for mutations. However, the specific effects of mutations in the (AdhE) and their impact on ethanol production remain unclear.

Approach

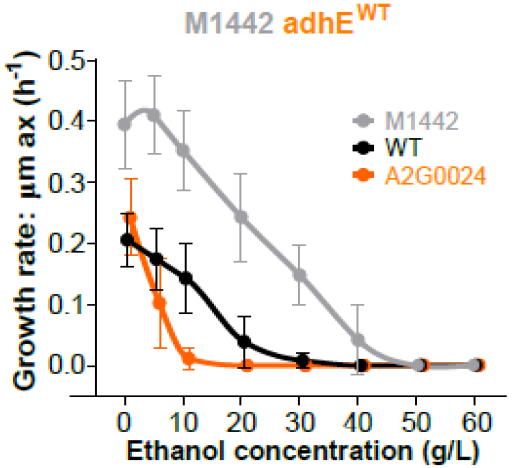
- To understand why AdhE is a frequent target of mutations in *T. saccharolyticum*, we combined microbial physiology experiments with biochemical enzyme assays.

Results

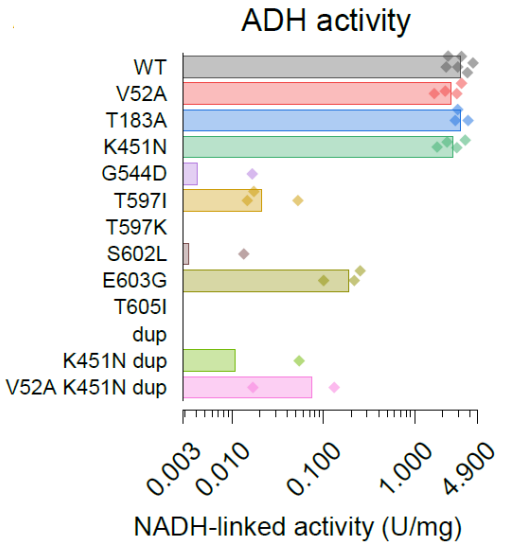
- Reintroducing wild-type (WT) AdhE into an ethanologenic strain reduced ethanol tolerance.
- NADH-linked alcohol dehydrogenase (ADH) activity was associated with ethanol sensitivity, indicating that eliminating this activity is key to achieving high ethanol titers.
- In the absence of NADH-linked ADH activity, a separate NADPH-linked enzyme compensates by providing NADPH-dependent activity.

Significance/Impacts

- Understanding how *T. saccharolyticum* achieves high-titer ethanol production is key to transferring this trait to other organisms.
- Strictly NADPH-linked ADH activity is important for high-titer ethanol production.
- These findings can inform engineering strategies for other ethanol production platforms, including *Clostridium* or *Zymomonas* species.



Reintroduction of the WT AdhE (NADH-linked) into an ethanologen strain of *T. saccharolyticum* decreased ethanol tolerance.



Characterization of changes in ADH activity of AdhE mutants. Mutants that decrease ADH activity are associated with increased ethanol tolerance.

Fabri J. et al. *J. Bacteriology* (2025) Vol. 207 No. 5 doi/10.1128/jb.00015-25