The Role of AdhE on Ethanol Tolerance and Production in C. thermocellum

Background

- An alcohol dehydrogenase, AdhE, catalyzes the conversion of acetyl-CoA to ethanol which allows bacteria to generate energy and maintain their metabolic activities in environments lacking oxygen. In this study, we aim to better understand why AdhE is a frequent target for mutations, how ethanol tolerance affects ethanol production and the underlying biochemical basis for these phenotypes in *Clostridum thermocellum*.

Approach

- To compare effects of specific mutations in *adhE*, we homogeneously expressed *adhE* mutants in a Δ*adhE* strain and measured their enzymatic activity, ethanol tolerance and product formation in *C. thermocellum*.
- To better understand how these AdhE mutations affected ethanol tolerance and fermentation behavior, we measured enzyme activity using AdhE mutant enzymes cloned and purified from *E. coli*.

Results

- There is a strong correlation between NADH-linked ADH activity and ethanol tolerance. Mutations that decrease NADH-linked ADH activity increase ethanol tolerance (Fig. 1); correspondingly, mutations that increase NADH-linked ADH activity decrease ethanol tolerance. The cofactor specificity of ADH activity was found to be the primary factor affecting ethanol yield.
- The magnitude of ADH activity did not play a significant role in determining ethanol titer (Fig. 2). Increasing ADH activity had no effect on ethanol titer. Reducing ADH activity had indeterminate effects on ethanol titer, sometimes increasing and sometimes decreasing it.

Significance

- The NADH-linked ADH activity of AdhE creates high ethanol sensitivity, accordingly the cell responds to this situation by mutating the ADH domain of AdhE. This is an important result for ethanol production in this organism: to avoid a tradeoff between ethanol tolerance and production, ADH activity should be NADPH-linked. These results will be used to understand AdhE's metabolic role and to guide metabolic engineering.

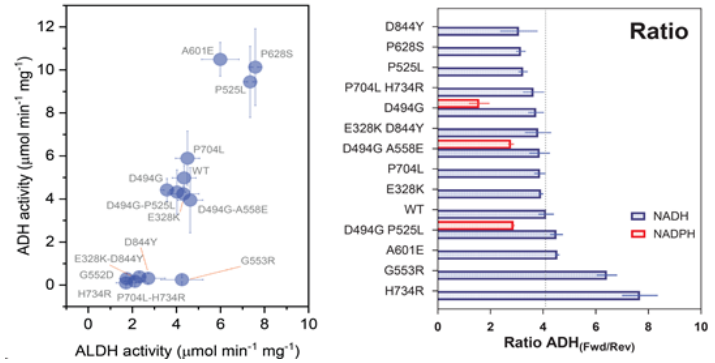

Figure 1. Comparison of enzyme activity of purified AdhE enzymes.

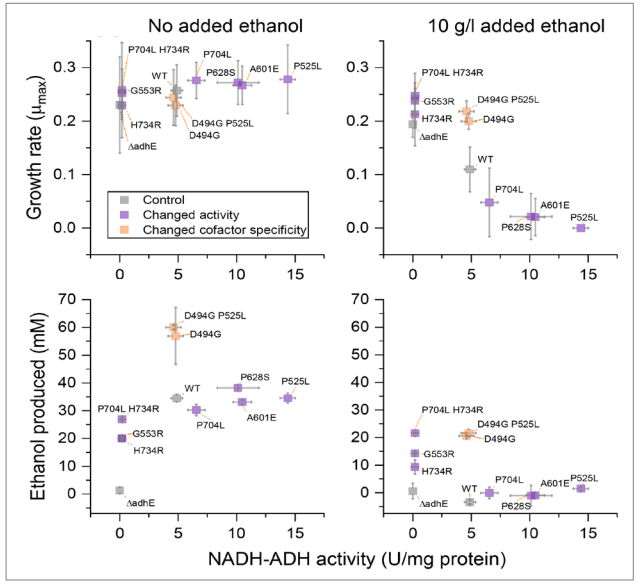

Figure 2. The effect of AdhE mutations on ethanol tolerance in *C. thermocellum*.

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