A Distinct Class of Ferredoxin:NADP$^+$ Oxidoreductase Enzymes Driving Thermophilic Ethanol Production

Background/Objective

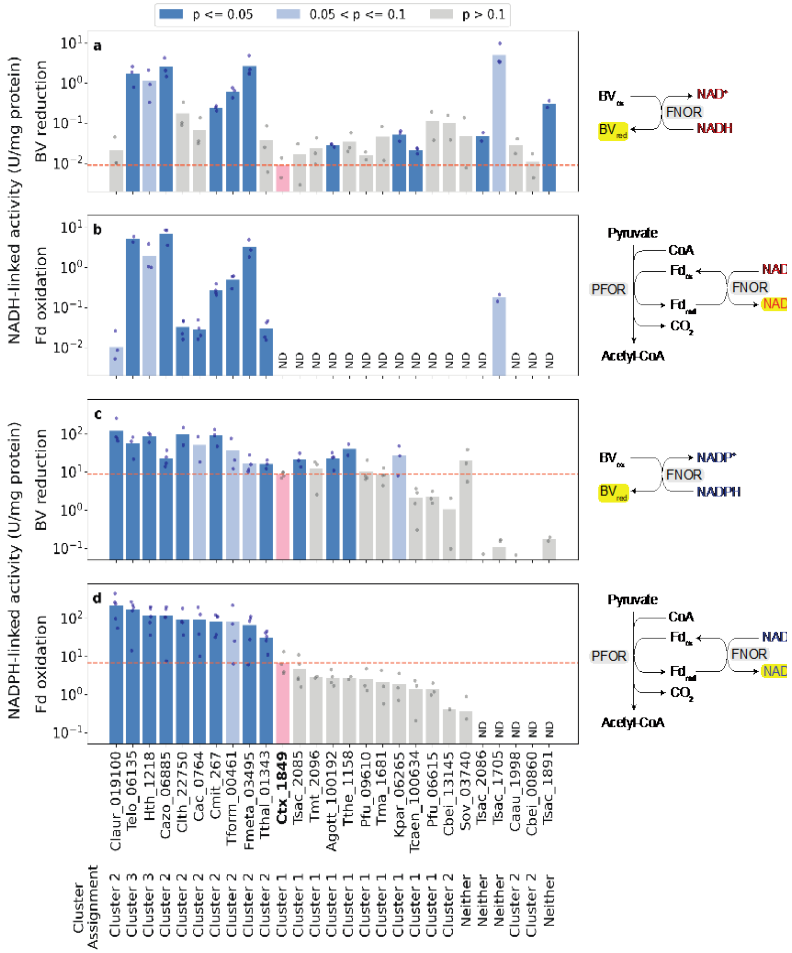
- Achieving high-titer ethanol production from lignocellulosic biomass in *Clostridium thermocellum* is limited by ethanol sensitivity linked to NADH-dependent alcohol dehydrogenase activity. This study aimed to identify thermostable ferredoxin:NADP$^+$ oxidoreductases (Fnors) to support a novel NADPH-linked ethanol production pathway.

Approach

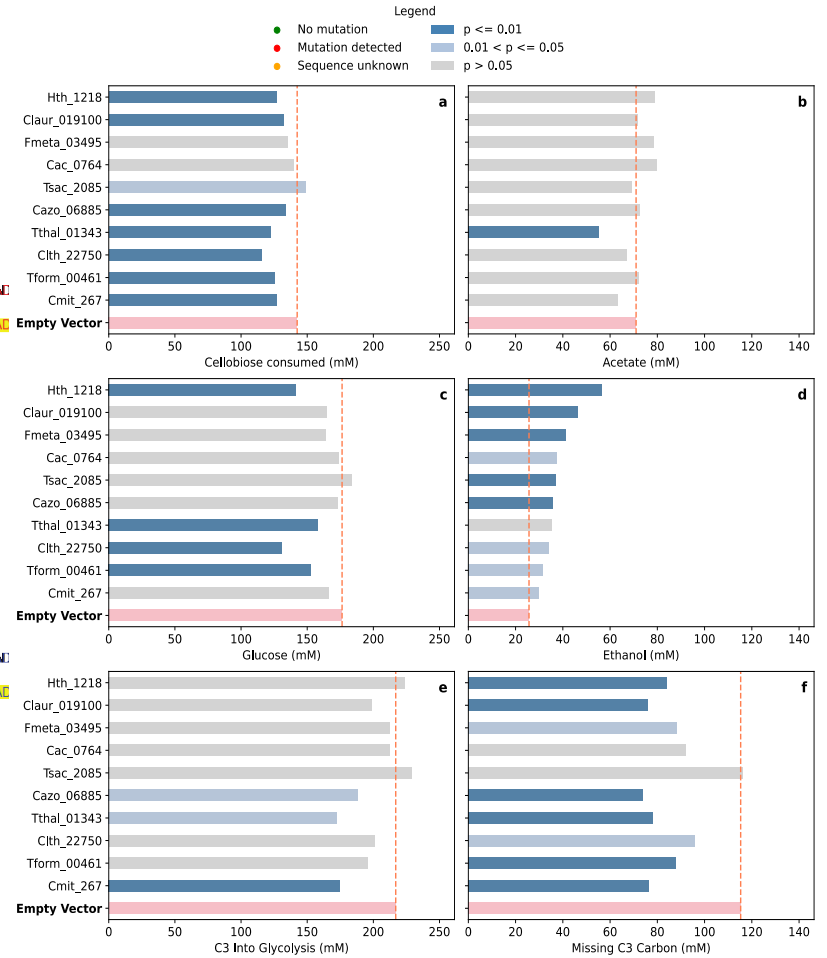
- We conducted biochemical characterization, including activity assays, with purified proteins and thermostability testing at 55°C, for 27 candidate enzymes. Promising Fnors were functionally expressed in *C. thermocellum* LL1784, followed by fermentation experiments to assess ethanol production.

Results

- We identified a novel class of Cac_0764-type Fnors, distinct from known NfnB proteins.
- Ten enzymes showed strong NADPH-linked activity, with four remaining thermostable at 55°C.
- Top candidates (Hth_1218 and Claur_019100) increased ethanol production up to 2.2-fold in *C. thermocellum* LL1784.



Enzymatic activities of the Fnor enzymes with various cofactors and electron acceptors/donors.



Impact of Fnor expression in *C. thermocellum* LL1784 on fermentation profiles.

Significance/Impacts

- This research establishes a new metabolic pathway for ethanol production that addresses key limitations in consolidated bioprocessing of lignocellulosic biofuels. The discovery of a novel Fnor enzyme class expands our understanding of microbial electron transfer and offers new tools for metabolic engineering beyond ethanol.

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