

Expressing the *Thermoanaerobacterium saccharolyticum* pforA in engineered *Clostridium thermocellum* improves ethanol production

Background

- Pyruvate:ferredoxin oxidoreductase (Pfor) is an important enzyme involved in the conversion of pyruvate to ethanol in thermophiles such as *Thermoanaerobacterium saccharolyticum* and *Clostridium thermocellum*. Engineered *T. saccharolyticum* uses PforA to produce ethanol at high yields (>80%) and titers (70 g/L). We sought to investigate whether introduction of *T. saccharolyticum* pforA into *C. thermocellum* improves ethanol production.

Approach

- We introduced *T. saccharolyticum* pforA into *C. thermocellum* and then evaluated its effects on fermentation performance to determine if engineered lines show improved ethanol production. We also explored whether the introduced *T. saccharolyticum* pforA can support ethanol production in the absence of native *C. thermocellum* Pfor enzymes by iteratively deleting the all five annotated pfor genes.

Results

- Expression of *T. saccharolyticum* pforA in *C. thermocellum* resulted in both improved ethanol yield and titer when fermentation was carried out using moderate solids loading (50 g/L cellobiose). Yields increased further when pforA was introduced in combination with other *T. saccharolyticum* genes, including *adhA*, *nfnAB*, and *adhE^{G544D}* (Fig. 1).
- At high solids loading (100 g/L Avicel), the effects of the introduced pforA gene on ethanol titer were less pronounced (Fig. 2).

Significance

- The introduction of pforA from *T. saccharolyticum* into *C. thermocellum* improved ethanol production.

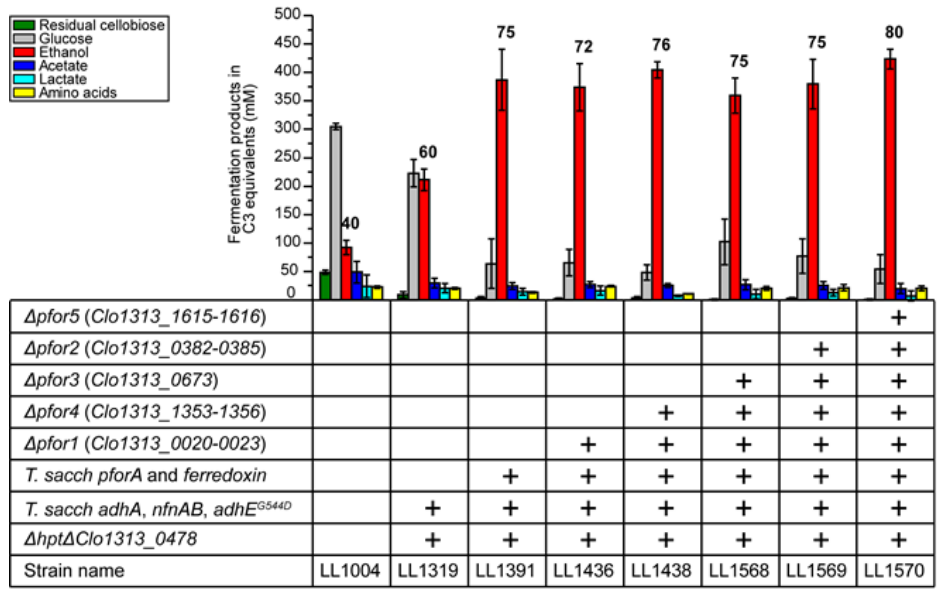


Figure 1: Fermentation products on 50 g/L cellobiose

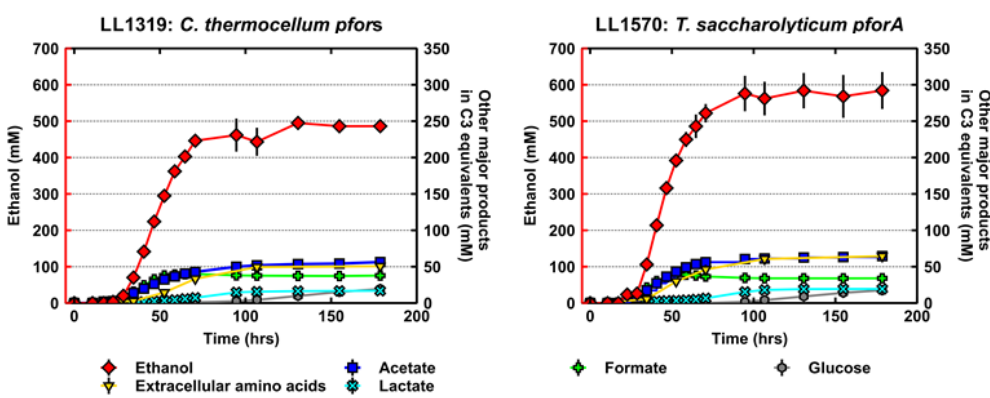


Figure 2: Fermentation products on 100 g/L Avicel

Hon S, Holwerda EK, Worthen RS, Maloney MI, Tian L, Cui J, et al. "Expressing the *Thermoanaerobacterium saccharolyticum* pforA in engineered *Clostridium thermocellum* improves ethanol production" *Biotechnol. Biofuels*. 11:242. 2018 doi:10.1186/s13068-018-1245-2.

