# Engineering *T. saccharolyticum* ethanol pathway into *C. thermocellum* improves biofuel production

U.S. DEPARTMENT OF ENERGY | Office of Science

## Background

- *Clostridium thermocellum* is a candidate for ethanol production from cellulose, but requires metabolic engineering to improve yield and titer.
- *Thermoanaerobacterium saccharolyticum* was previously engineered to produce ethanol at high yield and titer. Four of the genes that are responsible for ethanol production are *adhA*, *nfnA/B*, and *adhE*$^{G544D}$.

## Approach

- *T. saccharolyticum adhA*, *nfnAB*, and *adhE*$^{G544D}$ were introduced into wild type *C. thermocellum*, first on expression plasmids to do pathway optimization, then by integrating onto the chromosome.
- The introduced pathway was combined with hydrogenase deletions to determine whether this will lead to further improvements to ethanol production.

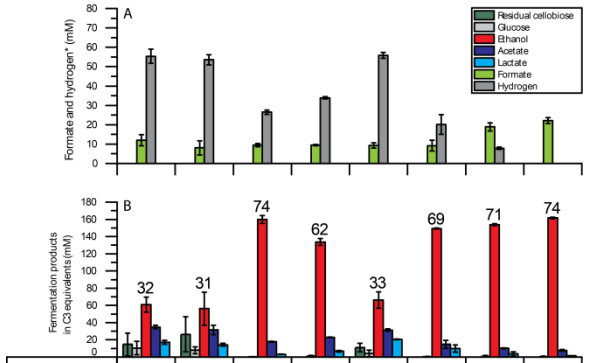
## Outcome

- Introducing the four *T. saccharolyticum* genes into *C. thermocellum* significantly increased ethanol yield.
- The maximum ethanol titer achieved by this engineering approach was 15 g/L.
- Hydrogenase deletions decreased ethanol titer.

## Significance

- The *T. saccharolyticum* ethanol production pathway is a promising strategy for improving ethanol production in *C. thermocellum*.

**adhA, nfnAB, and adhE$^{G544D}$ expression in *C. thermocellum***

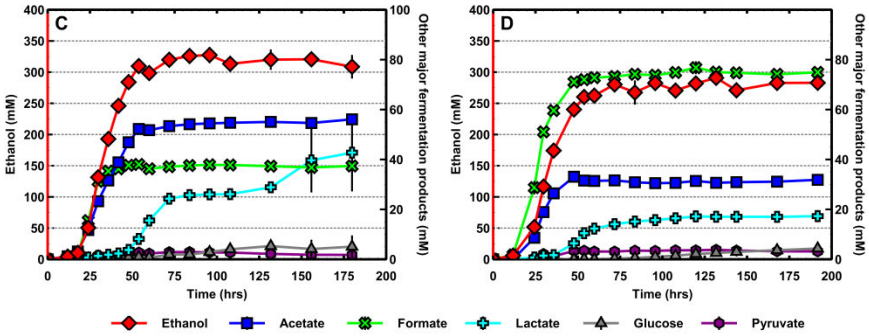



| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| *T. sacch adhA* | | | + | + | + | + | + | + |
| *T. sacch nfnAB* | | | + | + | + | + | + | + |
| *T. sacch adhE* | | | + | | + | + | + | + |
| *ΔC. therm adhE* | | | | | + | | | |
| *Δech* | | | | | | + | | + |
| *ΔhydG* | | | | | | | + | + |
| *ΔhptΔ0478* | | + | + | + | + | + | + | + |
| Strain name | LL1004 | AG929 | LL1319 | LL1390 | LL1323 | LL1324 | LL1325 | LL1381 |

**Fermentation on high substrate concentration (60 g/L Avicel)**

**LL1319: *C. thermocellum* + *T. saccharolyticum adhA, nfnAB, adhE*$^{G544D}$**

**LL1381: LL1319 with hydrogen production eliminated (*ΔhydGΔech*)**





Hon, S., Olson, D.G., Holwerda, E.K., Lanahan, A.A., Murphy, S.J., Marybeth, M., Zheng, T., Papanek, B.A., Guss, A.M., Lynd, L.R., 2017. "The ethanol pathway from *Thermoanaerobacterium saccharolyticum* improves ethanol production in *Clostridium thermocellum*." *Metab. Eng.* 42, 175–184. doi:10.1016/j.ymben.2017.06.011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