Increased Lactate Tolerance of *Clostridium thermocellum*

U.S. DEPARTMENT OF ENERGY | Office of Science

Background

- Lactic acid (LA) has applications in the food, cosmetics and pharmaceutical industries, as well as in the production of biodegradable plastic polymers. Recent reports have shown the potential of the cellulolytic bacterium *Clostridium thermocellum* for direct LA production from inexpensive lignocellulosic biomass. However, *C. thermocellum* is highly sensitive to acids and does not grow below pH ~6.0. Improvement of LA tolerance of this microorganism becomes a proof-of-principle for the application of adaptive laboratory evolution (ALE).

Approach

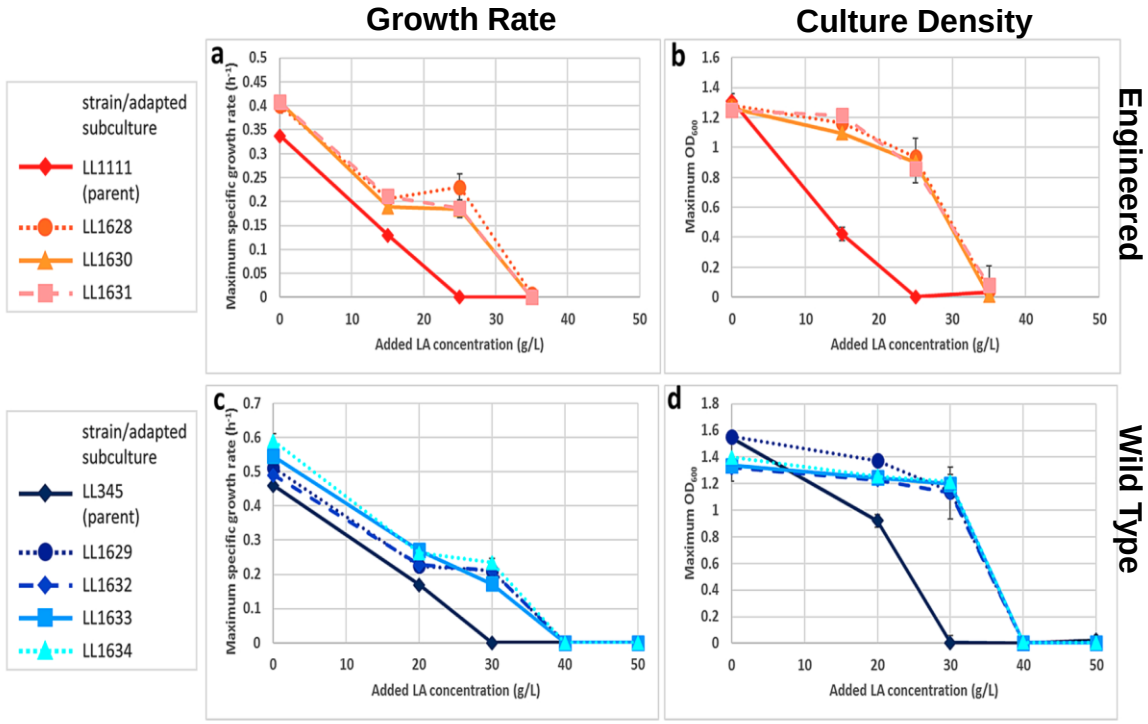
- We performed ALE on wild-type *C. thermocellum* (LL345) on a strain that had been engineered for increased lactate production through the elimination of the ethanol production pathway (LL1111)
- Evolved strains were resequenced at JGI and analyzed for mutations

Outcome

- After ALE on strain LL1111, the lactate tolerance had more than doubled, increasing from 15 g/L to 35 g/L. Mutations in the gene loci encoding PPDK (pyruvate-phosphate dikinase) and HPr K/P (HPr kinase/phosphorylase) suggest that some modification affected carbohydrate and/or phosphate metabolism

Significance

- Strains of *C. thermocellum* with improved lactate tolerance may be used as a platform for increased cellulosic lactate production. The identified mutations via ALE suggest targets for further understanding the mechanism of growth inhibition in C. therm is possible.



Evolved strains from both wild-type (c, d) and engineered strains (a, b) of *C. thermocellum* show improved growth (both growth rate and maximum culture density) after adaptive laboratory evolution in the presence of added lactate.

Mazzoli et al. "*In vivo* evolution of lactic acid hyper-tolerant *Clostridium thermocellum*." (2021) *New Biotechnology* 67: 12-22. doi: 10.1016/j.nbt.2021.12.003

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