Engineering Pyrophosphate-Free Glycolysis in Clostridium thermocellum

Background

- Sustainable biofuels, like ethanol, are essential for reducing carbon emissions in transportation. *Clostridium thermocellum* is a leading candidate for cellulosic ethanol production due to its robust lignocellulose metabolism. However, its native glycolysis pathway relies on pyrophosphate (PPi), creating thermodynamic bottlenecks that limit ethanol production. Achieving higher ethanol titers is critical for commercial feasibility.

Approach

- This research focused on engineering *C. thermocellum* to eliminate PPi-dependent glycolytic reactions, addressing thermodynamic bottlenecks. The PPi-dependent phosphofructokinase (PPi-PFK) was replaced with an ATP-dependent PFK to enhance the driving force of glycolysis. Additionally, pyruvate kinase (PYK) and a soluble pyrophosphatase (PPase) were introduced to establish a fully PPi-free glycolysis pathway. Fermentation experiments and metabolite analyses were conducted to assess the impact on ethanol titers and glycolytic flux.

Results

- Engineered strain exhibited a 38% increase in ethanol titer (from 15.1 g/L to 21.0 g/L).
- Enhanced thermodynamic driving force reduced reversibility of PFK reactions.
- Higher levels of lower glycolysis metabolites (e.g., PEP, 3PG) observed in the PPi-free strain.
- Demonstrated the feasibility of engineering pathway thermodynamics to improve product titers.

Significance

- This work demonstrates the potential of thermodynamic optimization as a powerful tool in metabolic engineering to enhance product titers. By transitioning *C. thermocellum* glycolysis to a canonical ATP-driven pathway, it establishes a foundation for further improving ethanol production. The results represent a significant step toward achieving ethanol titers required for commercial viability.

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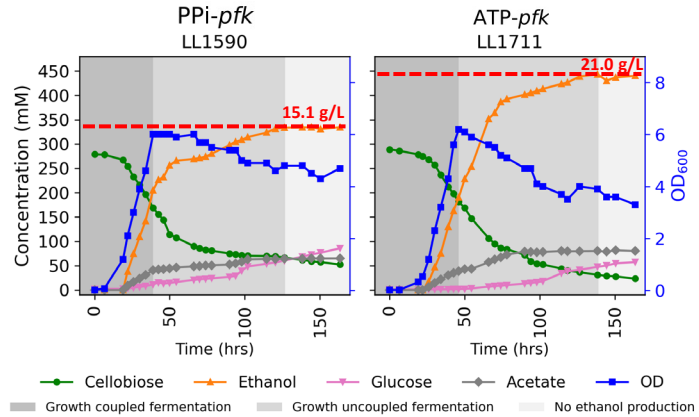
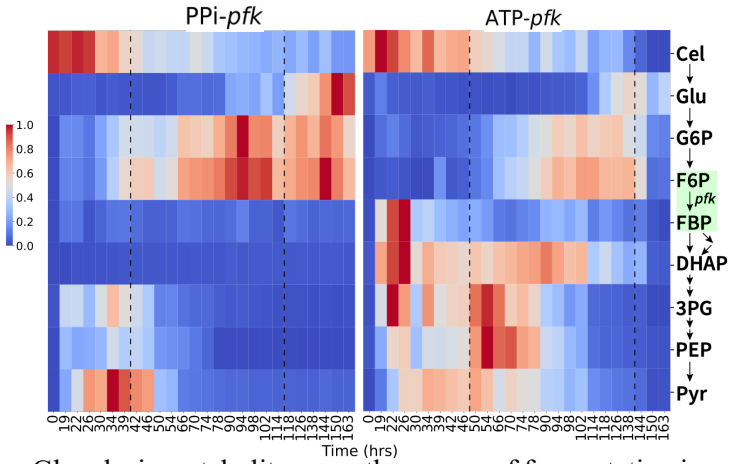


Batch fermentations of *C. thermocellum* strains with wild type glycolysis (strain LL1590) and PPi-free glycolysis (strain LL1711).



Glycolysis metabolites over the course of fermentation in PPi-*pfk* strain vs ATP-*pfk* strain

Key: cellobiose (Cel); glucose (Glu); glucose-6-phosphate (G6P); fructose-6-phosphate (F6P); fructose-1,6-bisphosphate (FBP); dihydroxyacetonephosphate (DHAP); 3-phosphoglycerate (3PG); phosphoenolpyruvate (PEP); pyruvate (Pyr)