Development of a genome-scale metabolic model of *Clostridium thermocellum* and its applications for integration of multi-omics datasets and computational strain design

U.S. DEPARTMENT OF ENERGY | Office of Science

Background

- The anaerobic thermophile *C. thermocellum* is a promising bacterium for bioconversion due to its capability to effectively degrade lignocellulosic biomass.
- However, the complex metabolism of *C. thermocellum* is not fully understood, hindering metabolic engineering to achieve high titers, rates, and yields of targeted biofuel or bioproducts.

Approach

- Built an updated genome-scale metabolic model of *C. thermocellum* that accounts for recent metabolic findings, improves prediction accuracy, and is standard-conformant to ensure easy reproducibility by UTK, PSU and ORNL.
- Utilized the model for elucidating complex redox metabolism of *C. thermocellum* from multi-omics datasets and enabling modular cell design.

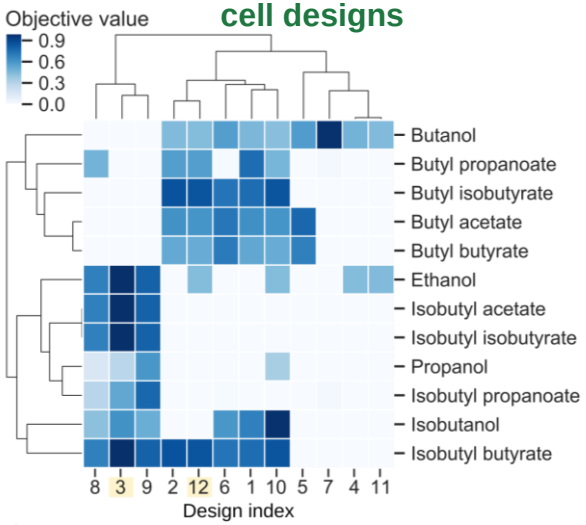
Outcomes and Impacts

- Developed the *C. thermocellum* model iCBI655 that reflects the most current genetic and metabolic knowledge and emphasizes accessibility and reproducibility through standard-conformance, interactive metabolic maps, and documentation.
- Trained iCBI655 against a comprehensive dataset of extracellular fluxes and known lethal genotypes and conditions, leading to enhanced prediction accuracy of growth phenotypes across culture conditions.
- Developed a multi-omics integration protocol and applied it to elucidate redox metabolism and potential redox bottlenecks critical for production of biofuels in *C. thermocellum* by using proteomics and extracellular flux datasets of the ethanol over-producing hydrogenase deletion mutants.
- Designed modular *C. thermocellum* production strains for effective biosynthesis of alcohols and esters in a growth-coupled manner that enables pathway optimization and selection by adaptive laboratory evolution.

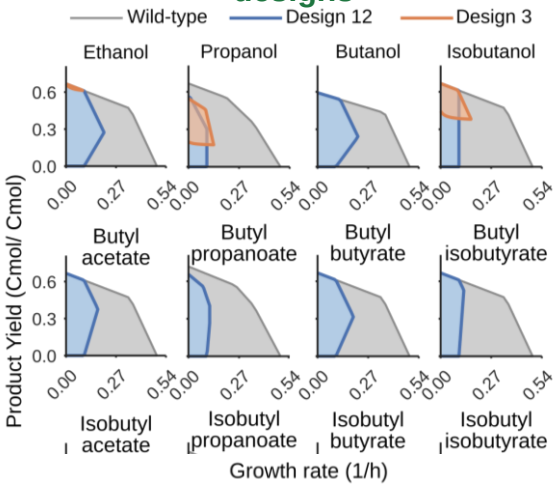
Significance

- Created a *C. thermocellum* metabolic model for interrogating its complex metabolism and enabling biocatalyst design via pathway optimization for efficient conversion of lignocellulosic biomass to biofuels.

Pareto front of various modular cell designs



Phenotypic spaces for representative designs



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Garcia, Thompson, Giannone, Dash, Maranas, Trinh, *Frontiers Bioeng Biotechnol,* accepted (2020)