Engineered enzyme allows direct isobutyl acetate ester directly from cellulose by *Clostridium thermocellum*

Background

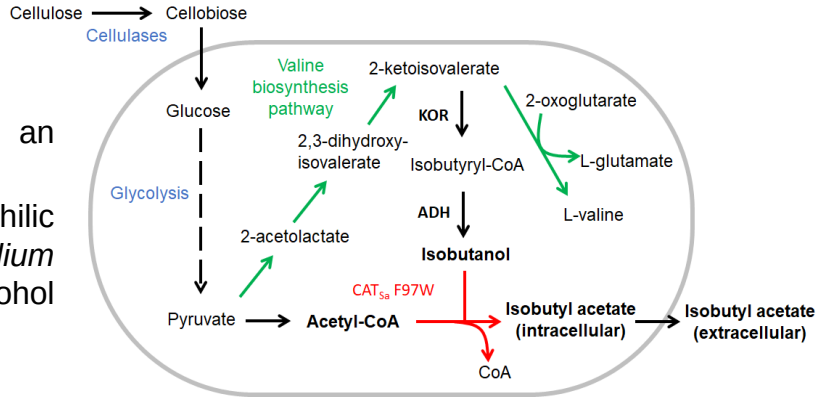
- Esters are versatile chemicals and potential drop-in biofuels.
- Volatility of esters may endow thermophilic bioproduction with an advantage in downstream product separation.
- Ester production directly from lignocellulosic biomass by the thermophilic consolidated bioprocessing (CBP) microbes, e.g., *Clostridium thermocellum* is limited by the availability of thermostable alcohol acetyltransferases (AATs).

Approach

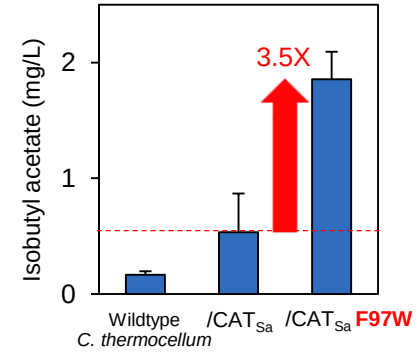
- Analyzed the alcohol substrate range of a thermostable chloramphenicol acetyltransferase from *Staphylococcus aureus* (CAT_{Sa}).
- Used model-guided protein engineering to identify the F97W mutation of CAT_{Sa} into an AAT responsible for enhanced isobutyl acetate production.
- Demonstrated direct conversion of cellulose into isobutyl acetate at high temperature in an engineered thermophilic *C. thermocellum.*

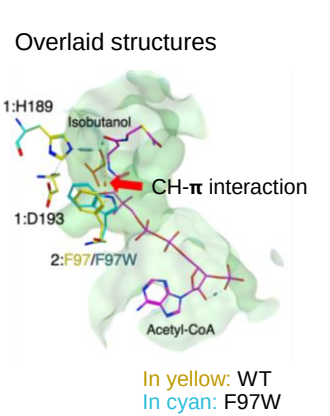
Outcomes and Impacts

- Discovered a broad alcohol substrate range of CAT_{Sa} through both *in silico* and *in vivo* characterization.
- Revealed that the F97W mutation of CAT_{Sa} can improve isobutyl acetate production using model-guided protein engineering.
- Demonstrated a CAT can function and/or be re-purposed as an AAT for novel biosynthesis of designer esters at elevated temperature.
- Presented the first report on CBP of cellulose into ester(s) by the thermophilic *C. thermocellum* engineered with thermostable CAT.



Biosynthesis of isobutyl acetate from cellulose in an engineered *C. thermocellum*





Characterization of CAT_{Sa} and its F97W variant *in vivo* (Panel A), *in vitro* (Panel B), and *in silico* (Panel C).

Significance

- Established a foundation for engineering non-model organisms for direct conversion of biomass into designer bioesters.

Seo, H., Lee, J-W., Garcia, S., and Trinh, C.T., "Single mutation at a highly conserved region of chloramphenicol acetyltransferase enables isobutyl acetate production directly from cellulose by Clostridium thermocellum at elevated temperatures", *Biotechnology for Biofuels*, 12:245, 2019. doi: 10.11186/s13068-019-1583-8