Engineering Promiscuity of Chloramphenicol Acetyltransferase for Microbial Designer Ester Biosynthesis

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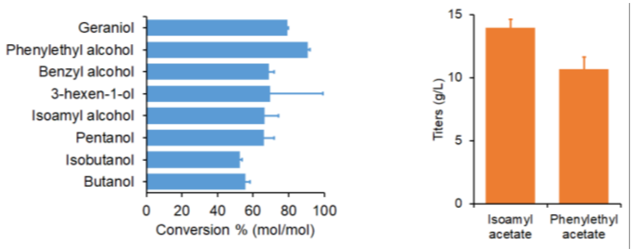
Background

- By condensing an acyl-CoA and an alcohol, alcohol acyltransferases (AATs) can serve as an interchangeable metabolic module for microbial biosynthesis of a diverse class of ester molecules with broad applications as flavors, fragrances, solvents, and drop-in biofuels.
- The current lack of robust and efficient AATs limits their utility with precursor pathways and microbial hosts.

Approach

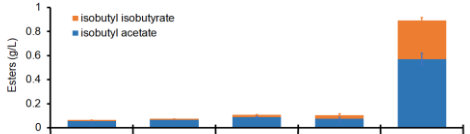
- Through bioprospecting and model-guided protein engineering, we engineered substrate promiscuity of chloramphenicol acetyltransferases (CATs) to function as robust and efficient AATs compatible with at least 21 alcohols and 8 acyl-CoAs for microbial biosynthesis of linear, branched, saturated, unsaturated and/or aromatic esters in mesophiles and thermophiles.



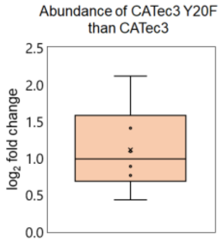
Demonstration of efficiency and compatibility of the engineered AAT in *Escherichia coli* whole-cell biocatalyst

Outcomes and Impacts

- Developed de novo thermostable AATs that are efficient and compatible with various pathways and microbial hosts (e.g., mesophiles and thermophiles).
- Demonstrated high conversion of various alcohols and achieved about 14 g/L of isoamyl acetate with >95% (mol/mol) conversion efficiency.
- Demonstrated that CAT robustness with enhanced thermostability is critical for efficient ester production in thermophiles by maintaining high level of intracellular CAT abundance.
- Engineered *C. thermocellum* to produce up to 1 g/L of isobutyl esters from cellulose.



	HSCT2105	HSCT2106	HSCT2113	HSCT2107	HSCT2108
CAT	CATsa F97W	CATsa Y20F	CATsa Y20F A138T	CATec3	CATec3 Y20F
Fold change	1.00	1.16	1.69	1.65	13.97
Melting point (ºC)	68.3 ± 1.2	69.3 ± 0.5	76.0 ± 1.2	80.2 ± 1.5	87.5 ± 0.5
k_{cat}/K_M (1/M/s)	4.1+/-0.7	10.5 ± 1.1	10.3+/-1.2	4.2 ± 0.1	13.0 ± 0.2



Impact of enzyme thermostability in ester production by *C. thermocellum* fermenting cellulose

Significance

- This work not only presents a robust, efficient, and highly compatible AAT platform for designer bioester production, but also elucidates the impact of enzyme thermostability on engineering heterologous pathways in thermophiles.

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