A High-Throughput Screening Platform Probes Enzymes for Designer Ester Biosynthesis

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Background

- Alcohol acyltransferases (AATs) enable microbial biosynthesis of many esters from an alcohol and an acyl-CoA. However, substrate promiscuity of AATs prevents microbial biosynthesis of designer esters with high selectivity.
- A high-throughput (HTP) screening method is needed to rapidly identify novel AATs for designer ester biosynthesis.

Approach

- We established a microplate-based culturing technique with *in situ* fermentation and extraction of esters and tested it by rapid profiling of alcohol substrate specificity of 20 chloramphenicol acetyltransferase variants derived from *Staphylococcus aureus*. This was coupled with a colorimetric assay.

Outcomes and Impacts

- We developed a HTP microbial screening platform for rapid profiling of the substrate preference of AATs that is scalable and compatible with automated microplate handling systems.
- This HTP screening platform probed the substrate specificity of both native and engineered AATs, It also identified the beneficial mutations in engineered AATs for enhanced ester synthesis.

Significance

- The high-throughput microbial screening platform developed provides a useful tool to rapidly identify novel wildtype and/or engineered AATs that have important roles in nature and industrial biocatalysis for designer bioester production.

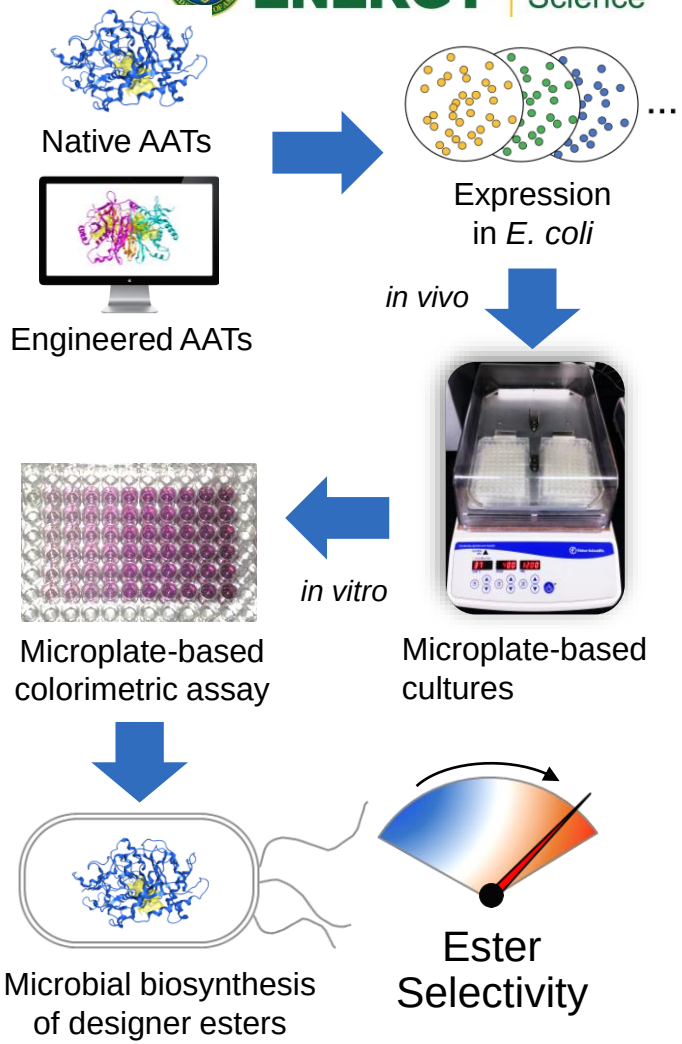

Fig. 1. A schematic workflow of the high-throughput microbial screening platform for rapidly probing specificity of AATs for biosynthesis of designer esters.

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Lee, J-W., Seo, H., Young C.,Trinh, C.T., *Biotechnol. Bioeng.*, 2021. https://doi.org/10.1002/bit.27926