Controlling Selectivity of Modular Microbial Biosynthesis of Butyryl-CoA-Derived Designer Esters

U.S. DEPARTMENT OF ENERGY | Office of Science

Background

- The *de novo* microbial biosynthesis of a large library of esters as drop-in fuels and high-valued chemicals from fermentable sugars can be achieved by using an alcohol acetyltransferase (AAT) to condense endogenous acyl-CoAs with an alcohol.
- However, poor solubility and specificity of AATs together with insufficient generation of metabolite precursors present an outstanding metabolic engineering problem in controlling the biosynthesis of designer esters.

Approach

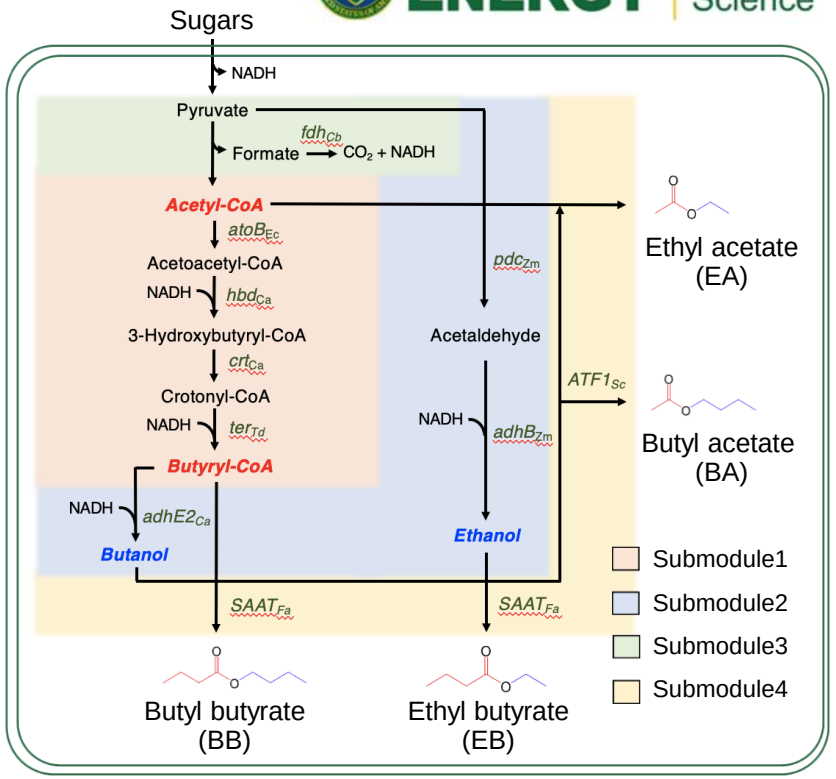
- We applied modular design principles to engineer the butyryl-CoA-derived ester pathways as exchangeable production modules.
- We screened a library of target ester production modules to identify the best ester production modules of butyryl-CoA-derived esters through manipulating replication (i.e., plasmid copy number), transcription (i.e., promoters), (post)translation (i.e., codon optimization), pathway enzymes, and pathway induction conditions.
- We implemented various protein solubilization strategies on aggregate-prone pathway enzymes to improve selective ester production.

Outcome

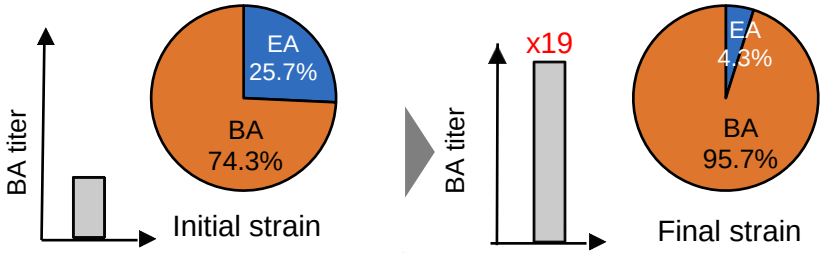
- We engineered *de novo* modular fermentative pathways of biosynthesis of butyl acetate (BA), ethyl butyrate (EB), and butyl butyrate (BB) from fermentable sugars.
- We achieved 19-fold increase in BA production (0.64 g/L, 96% selectivity), 6-fold increase in EB production (0.41 g/L, 86% selectivity), and 13-fold increase in BB production (0.45 g/L, 54% selectivity), as compared to the initial strains.

Significance

- This study presents a generalizable framework to enable the *de novo* fermentative microbial biosynthesis of butyryl-CoA-derived designer esters (e.g., butyl acetate, ethyl butyrate, butyl butyrate) with controllable selectivity from renewable biomass feedstocks. This highlights the power of implementing modular design principles for rapid strain engineering to harness a large space of designer esters from renewable feedstocks with broad utility.



Modular biosynthesis pathways of C4-derived esters. Submodule1-4 contains butyl-CoA synthesis, alcohol synthesis, NADH regeneration, and ester synthesis pathway, respectively.



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