Proteome Reallocation Enables the Selective de novo Biosynthesis of Non-Linear, Branched-Chain Acetate Esters

Background

- Short-chain esters are biobased chemicals used as flavors, fragrances, solvents, and biofuel additives.
- The one-carbon recursive ketoacid elongation pathway is important for making various branched-chain amino acids, ketoacids, aldehydes, alcohols, and acetate esters in living cells (**Panel A**).
- Controlling pathway selectivity to make one desired molecule (e.g., isoamyl acetate) is challenging due to enzyme promiscuity, regulation, and metabolic burden, requiring multi-level pathway engineering.

Approach

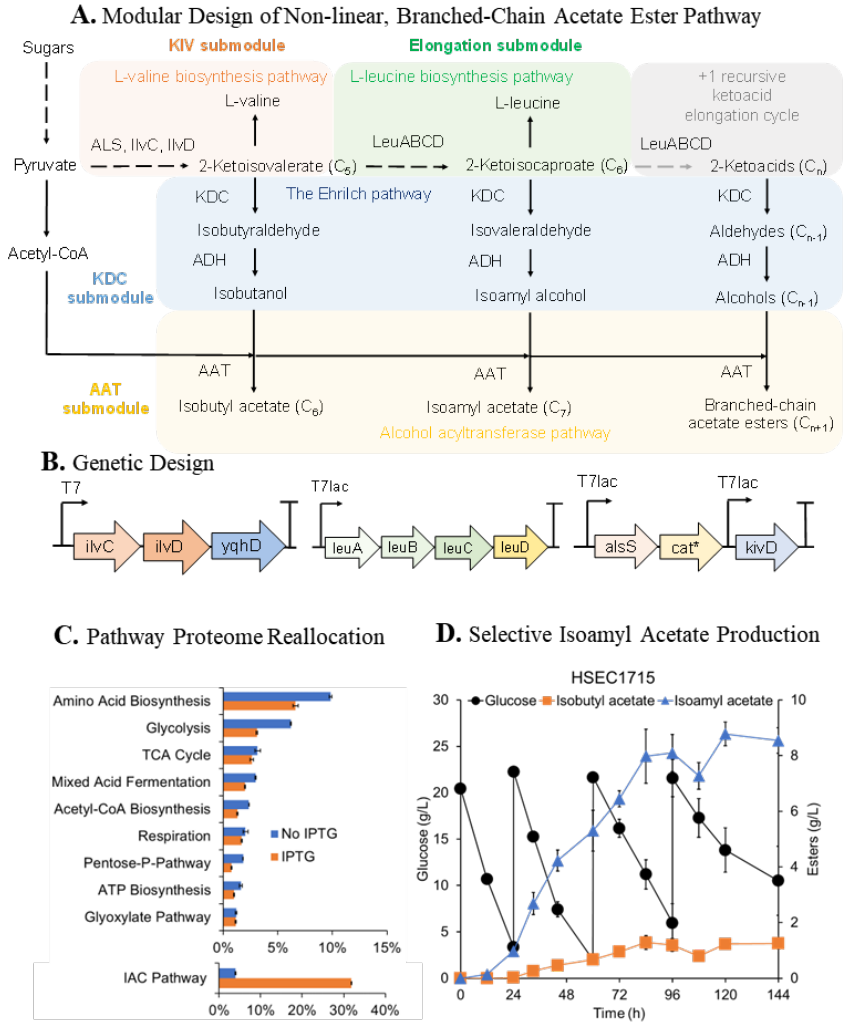
- Implemented pathway modularization for rapid and systematic design, construction, characterization, and optimization by partitioning the branched-chain ester pathways into four submodules: keto-isovalerate, ketoacid elongation, ketoacid decarboxylase, and alcohol acyltransferase (**Panels A&B**).
- Reallocated the pathway proteome to enable selective ester biosynthesis by systematic manipulation of pathway gene replication and transcription, enzyme specificity of the first committed steps of these submodules, and downstream competing pathways.

Results

- Presented a modular design framework to enable the *de novo* biosynthesis for designer, non-linear, branched-chain, acetate esters (**Panels A&B**).
- Demonstrated that controlling protein expression and specificity of the first committed steps of the four submodules is critical to achieve selective microbial biosynthesis of branched-chain acetate esters.
- Showed the optimized isoamyl acetate pathway globally redistributed the amino acid fractions in the proteomes and required up to 23-31% proteome reallocation at the expense of other cellular resources (**Panel C**).
- Created the engineered strains that could produce isoamyl acetate up to a titer of 8.8 g/L, a yield of 0.22 g/g, and 86% selectivity, achieving the highest titers, yields and selectivity of isoamyl acetate reported to date (**Panel D**).

Significance

- Proteome reallocation enables the selective *de novo* biosynthesis of non-linear, branched-chain acetate esters and shows potential metabolic tradeoffs.



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