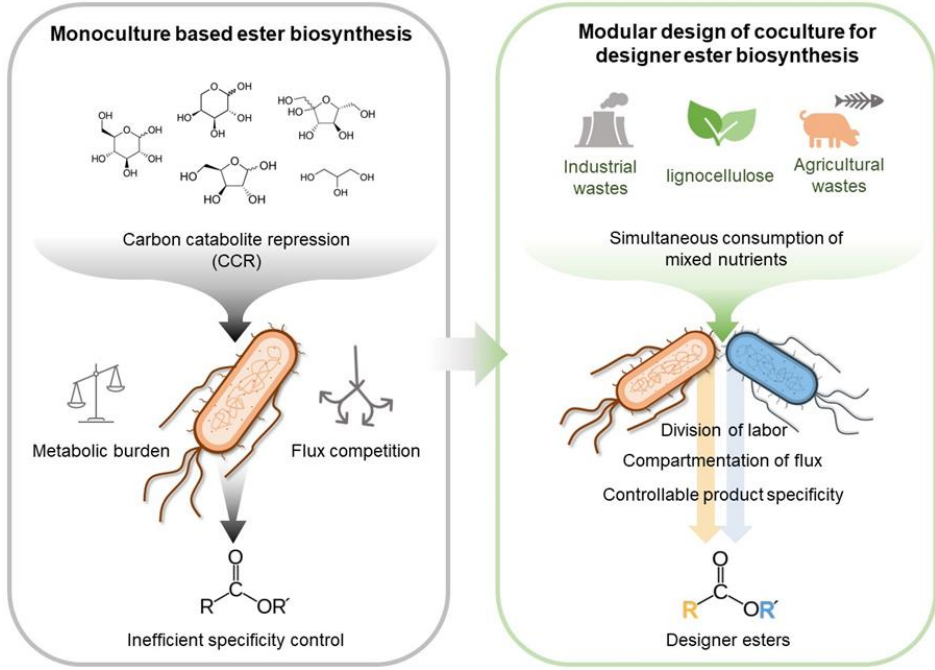
Engineering a synthetic Escherichia coli coculture for compartmentalized de novo biosynthesis increases production of esters from biomass mixed sugars

Background

- Short-chain esters are versatile chemicals with broad use as flavors, fragrances, solvents, and fuels.
- *De novo* ester microbial biosynthesis of complex ester molecules such as isobutyl butyrate consists of complex metabolic pathway submodules, which is challenging to engineer to achieve optimal metabolic fluxes and selective product synthesis.

Approach

- We compartmentalized the pathway submodules into specialist cells that facilitate pathway modularization and labor division for de novo ester biosynthesis from various renewable feedstocks.
- We engineered a synthetic *Escherichia coli* coculture with compartmentalized sugar utilization and ester biosynthesis pathways to produce isobutyl butyrate from a mixture of glucose and xylose. Specifically, the isobutyl butyrate pathway is modularized into the isobutanol, butyl-CoA, and ester condensation submodules where the isobutanol submodule is distributed to the glucose-utilizing specialist and the other submodules to the xylose-utilizing specialist.
- The xylose-utilizing *E. coli* specialist selectively consumes xylose over glucose and bypasses carbon catabolite repression (CCR) while leveraging the native CCR machinery to activate the glucose-utilizing *E. coli* specialist.
- We varied inoculum ratios of the two specialists and pH to increase metabolic flux to isobutyl butyrate



Results

- The importance of engineering CCR demonstrated in microbial coculture design for division of labor in mixed sugar use.
- We demonstrated a robust synthetic coculture selectively produces isobutyl butyrate with a 31-fold titer improvement as compared to the monoculture.
- We determined bottlenecks of selective isobutyl butyrate biosynthesis using synthetic cocultures.

Significance

- This illustrates that ester pathway compartmentalization is a generalizable strategy for harnessing modular microbial cocultures to effectively produce designer esters derived from complex diverging-converging metabolic pathways.
- We addressed metabolic engineering challenges of metabolic burden and flux competition by a synthetic microbial consortium.
- We identified CCR as a crucial factor affecting the type of microbial symbiosis.

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