Controlling Selectivity of Modular Microbial Biosynthesis of Designer Acetate Esters through Proteome Reallocation

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Project Goals: To fundamentally understand and redirect metabolism and regulation of thermophilic *Bacillus coagulans* for the efficient conversion of undetoxified lignocellulosic biomass hydrolysates into designer bioesters.

Abstract text: Short-chain esters have broad utility as ingredients for flavors, fragrances, solvents, and drop-in biofuels. Biologically, these esters are derived from the condensation of acyl CoAs and alcohols from cellular metabolism, resulting in a large portfolio of ester molecules. However, controlling the selectivity of microbial ester biosynthesis has remained challenging from a metabolic engineering standpoint. Here, we present a generalizable framework for the *de novo* biosynthesis of short-chain designer bioesters (i.e., n-butyl acetate, isobutyl acetate, and isoamyl acetate) through microbial fermentation with controllable selectivity from renewable feedstocks. Using modular design principles, we propose to design and package efficient ester production pathways into exchangeable ester modules compatible with an engineered chassis cell. Successful implementation of our strategy required manipulation of replication, transcription, (post)translation, and enzyme specificity to control proteome reallocation for designer ester biosynthesis. By coupling the exchangeable ester modules with the chassis cell(s), we demonstrated the assembled production strains exhibited enhanced production of target esters with high selectivity.

References/Publications

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