Harnessing Natural Diversity of Enzymes to Improve Metabolic Flux

U.S. DEPARTMENT OF ENERGY | Office of Science

Background

- Bacterial biocatalysts can transform lignin-derived aromatics to value-added bioproducts, thereby enabling a competitive bio-based economy.
- However, bottlenecks within the metabolic pathway limit the overall productivity and, by extension, process economics.

Approach

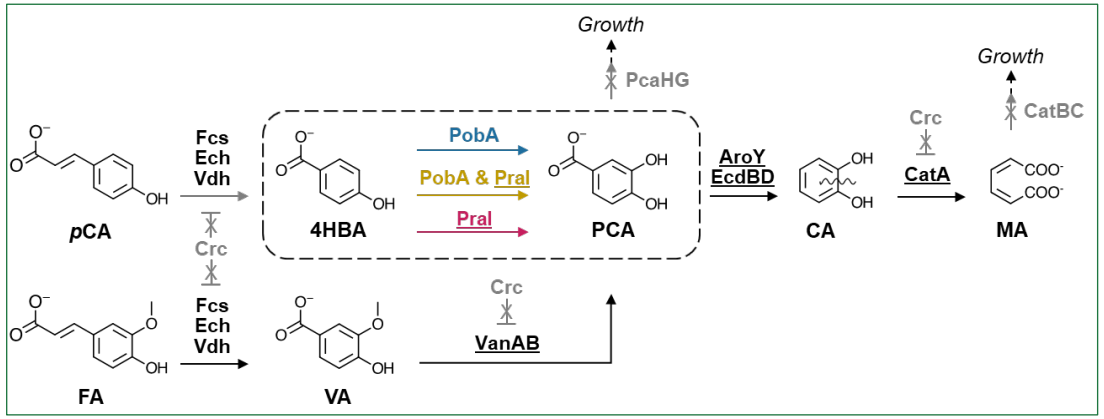
- Here we improve the metabolic flux from *p*-coumarate to muconate by harnessing the natural enzyme diversity, specifically in the conversion of 4-hydroxybenzoate to protocatechuate.
- We hypothesized that the substitution of the native *Pseudomonas putida* 4-hydroxybenzoate hydroxylase *pobA* with the non-native *pral* — which performs the same conversion with a broader cofactor specificity — would improve the flux.

Outcome

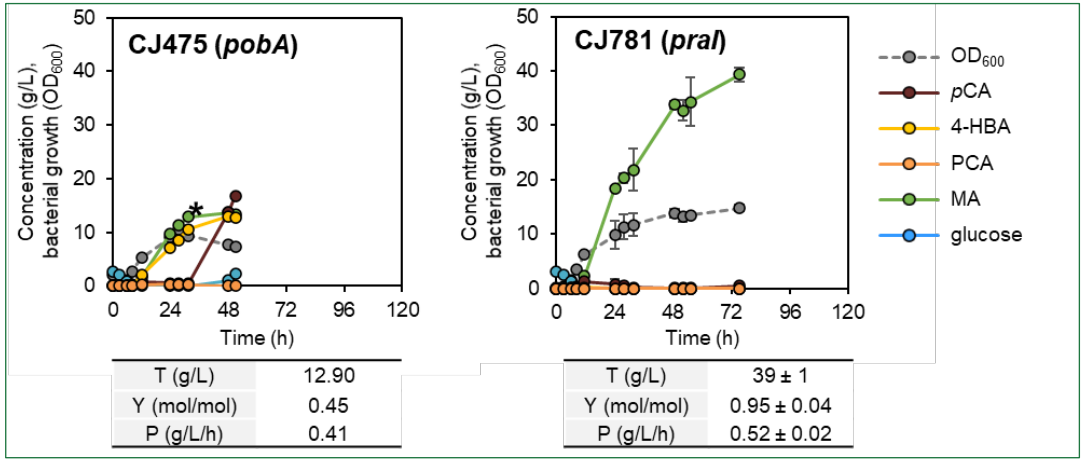
- The substitution alleviated the 4-hydroxybenzoate accumulation; the resulting strain achieved a titer of 40 g/L muconate at 100% molar yield and a productivity of 0.5 g/L/h using *p*-coumarate and glucose as feed.

Significance

- Substituting a rate-limiting enzyme debottlenecked the overall conversion.
- Improved productivities for biological lignin valorization works towards realizing economic viability based on process models.
- Simultaneously, this study provides insight into the cofactor specificity of flavo-oxygenases.



Metabolic pathway for the conversion of *p*-coumarate and ferulate to muconate in *Pseudomonas putida*. Chemical abbreviations: FA, ferulate; pCA, *p*-coumarate; VA, vanillate; 4-HBA, 4-hydroxybenzoate; PCA, protocatechuate; CA, catechol; MA, muconate.



Bioreactor cultivation comparing the performance of *P. putida* bearing either *pobA* (CJ475) or *pral* (CJ781) fed 9 mmol/h *p*-coumarate. Abbreviations: T, titer; Y, yield; P, productivity. Chemical abbreviations as above.

Kuatsjah, E et al. "Debottlenecking 4-hydroxybenzoate hydroxylation in *Pseudomonas putida* KT2440 improves muconate productivity from p-coumarate" Metabolic Engineering, 2022, 70: 31-42, DOI: 10.1016/j.ymben.2021.12.010

THE CENTER FOR BIOENERGY INNOVATION