Common mutations which improves poor aromatic-degrading enzymes function after transfer into a new bacterial host

Background

- When engineering microbes to introduce new metabolic pathways, the heterologous enzymes often function poorly.
- A typical approach is to screen multiple enzyme homologs in the hopes of identifying a variant that works immediately.
- Instead, we sought to understand why certain homologs were suboptimal, and what modifications were required for full activity.

Approach

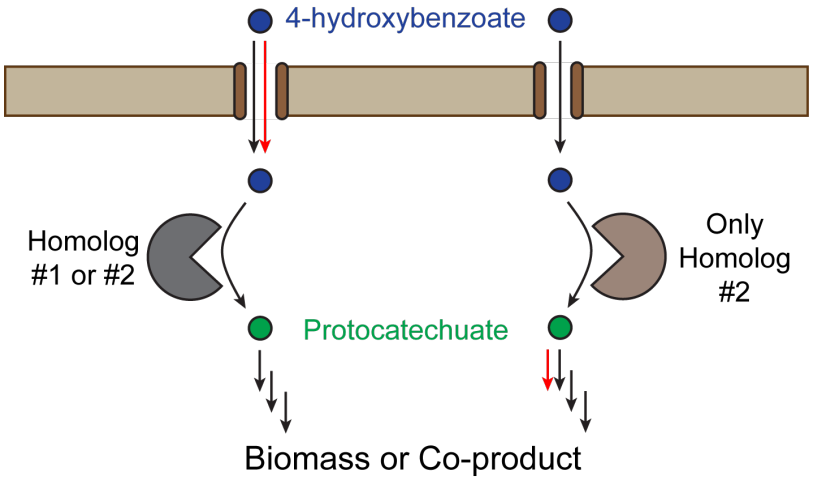
- Enzymes for 4-hydroxybenzoate (4-HB) catabolism were expressed in *E. coli*.
- Poorly-functioning enzymes were evolved *in vivo* to increase activity.
- Causes were identified by genetics and physiological measurements.

Outcomes

- Different enzyme homologs required alternate mutations to increase activity.
- All homologs required silent mutations that increased expression by destabilizing mRNA secondary structure.
- Mutations to the 4-HB transporter, which increased 4-HB imports, were beneficial in all cases.
- Increasing expression of downstream enzymes was sufficient for only one enzyme homolog.

Significance

- Understanding the factors that may limit function of heterologous enzymes will simplify the process of metabolic engineering.
- This 4-HB pathway is of particular interest for the conversion of lignin-derived aromatic compounds into value-added co-products.



Enzyme homologs required different modifications to support growth. Two enzyme homologs for the conversion of 4-HB to protocatechuate were expressed in *E. coli*. Multiple mutations were required to enable growth with 4-HB. Both enzymes required silent mutations that increased enzyme expression. Additional mutations that increased 4-HB import (red arrow, left) supported growth using either enzyme homolog. Mutations that increased PCA metabolism (red arrow, right) were only sufficient for one homolog.

Standaert RF, Giannone RJ, and Michener JK, Identification of parallel and divergent optimization solutions for homologous metabolic enzymes. *Metabolic Engineering Communications*, 6, 2018.

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