Optimizing Vanillate Catabolism in Pseudomonas putida KT2440

Background

- Depolymerization of lignin, followed by bioconversion in *Pseuduomonas putida* KT2440, is a promising approach to convert lignin to valuable products.
- Methoxylated monomers such as vanillate must undergo *O*-demethylation to enter catabolic pathways.
- Several enzyme families catalyze aromatic *O*-demethylation, but they are rarely compared *in vivo* to determine an optimal biocatalytic strategy.

Approach

- Vanillate *O*-demethylation pathways were compared by overexpression in engineered strains of *P. putida*, focusing on the distinct LigM and VanAB systems (**Figure 1**).
- Adaptive laboratory evolution optimized each pathway; mutations were identified by next-generation sequencing and investigated with enzyme kinetics and transcriptomics.

Results

- All evolved strains displayed improved growth phenotypes (**Figure 2**), but those harboring the optimized VanAB pathway demonstrated faster vanillate utilization.
- Mutations related to the VanAB pathway, including those in *vanB*, PP_3494 (a global regulator), and *fghA*, were investigated in greater detail to identify their contributions toward enhanced growth.
- These three mutations were reverse-engineered into a *P. putida* to generate an improved biocatalyst for the valorization of lignin.

Significance

- We illuminate the details of vanillate catabolism in the context of two distinct pathways.
- Rational engineering combined with adaptive evolution yields a platform strain for efficient conversion of vanillate to value-added products with implications for extension to other lignin monomers.

Bleem, A.C., *et al. Metabolic Engineering* (2024), 10.1016/j.ymben.2024.06.009

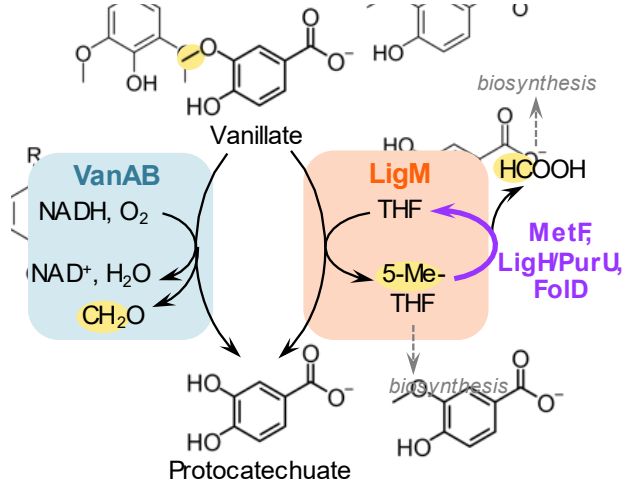

Figure 1. Two unique mechanisms for vanillate *O*-demethylation were investigated in *P. putida*

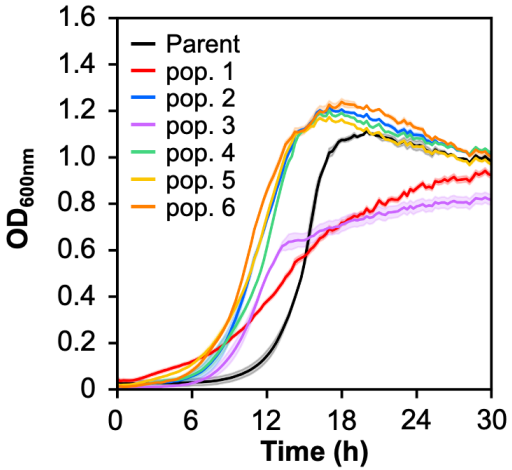

Figure 2. Evolved populations (colors) exhibited improved growth with vanillate, compared to the parent strains (black).