

Rapidly identifying new pathways for lignin valorization from non-model microbes

Background

- Cost-effective lignocellulosic biofuels will require producing valuable co-products from lignin.
- Lignin depolymerization produces a complex mixture of products.
- Biological valorization of this mixture will require engineering a microbe that can catabolize all of the various components.
- We do not currently know of pathways for catabolism of many lignin-derived aromatic compounds. New techniques to rapidly identify these pathways in non-model microbes will be critical.

Approach

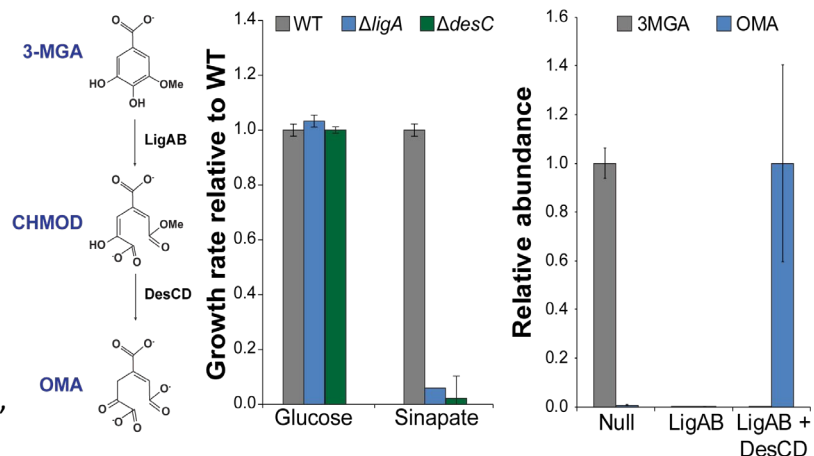
- We used transposon-insertion sequencing in a non-model microbe (*Novosphingobium aromaticivorans*) by using barcoded pooled libraries and measuring the depletion of knockouts which are no longer able to catabolize the aromatic substrate.
- After identifying novel aromatic-catabolizing enzymes, enzyme activities were confirmed by feeding experiments and heterologous expression.

Outcomes

- Transposon insertion sequencing can be used to differentiate redundant enzymes and pathways, decompose long pathways into short sub-networks, and rule out genes that might be involved in a particular phenotype.
- We identified and characterized a new pathway for catabolism of compounds derived from S-lignin.

Significance

- Our approach can be used to quickly identify entire catabolic networks from non-model microbes.
- Applications include further pathway discovery using the existing transposon library, as well as extension to new non-model microbes.



Discovery of new enzymes for catabolism of S-lignans. We identified a new pair of enzymes, which we named DesC and DesD, that provide a new route for bioconversion and valorization of lignin-derived aromatic compounds. Deletion of the genes prevents growth with S-lignans such as sinapate. Heterologous expression of the genes converts the substrate, 3-MGA, into the desired product, OMA.