

# Gene Fitness Profiling Elucidates Aromatic Catabolism in bacterium *Spingobium sp. SYK-6*

## Background

- Bioconversion of lignin-related aromatic compounds relies on robust catabolic pathways in microbes. *Spingobium sp. SYK-6* (SYK-6) is a well-characterized aromatic catabolic organism that has served as a model for microbial lignin conversion, and its utility as a biocatalyst could potentially be further improved by genome-wide metabolic analyses.

## Approach

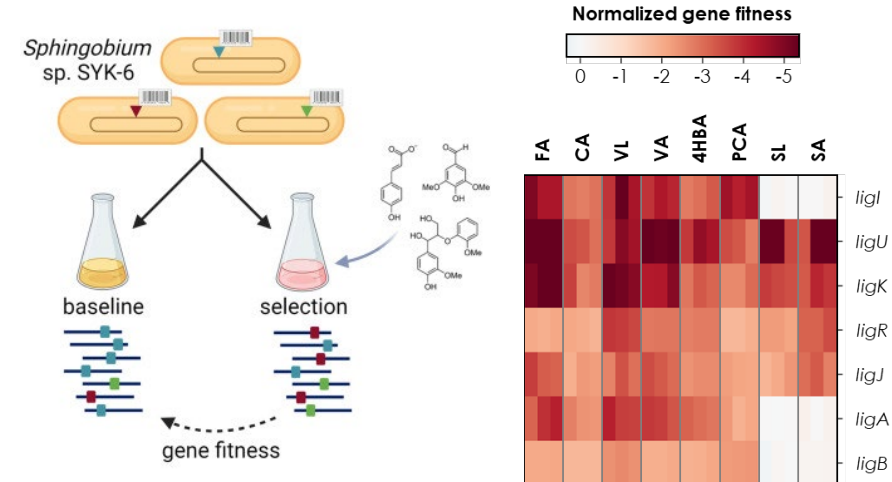
- We generated a randomly barcoded transposon insertion mutant (RB-TnSeq) library to study gene function in SYK-6. The library was enriched under various conditions (i.e., model lignin monomers and dimers, and acetic acid stress) to quantify gene fitness.
- Selected genes were further characterized by disrupting genes in SYK-6 or by heterologous expression in *Pseudomonas putida* KT2440

## Results

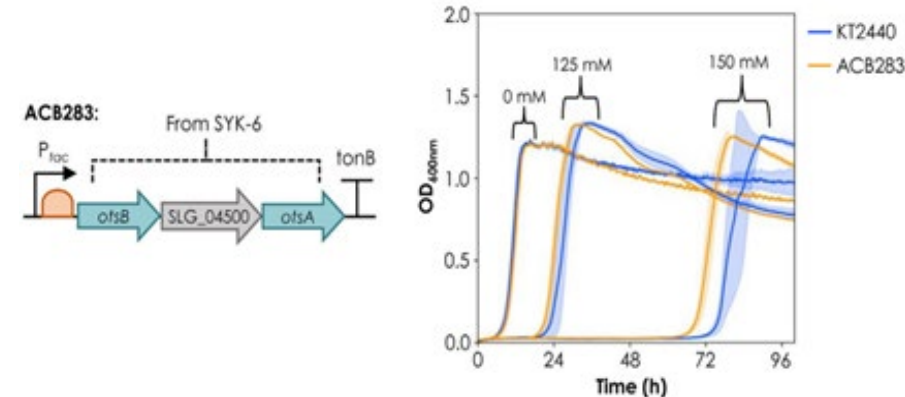
- Validated pathways for aromatic catabolism including biphenyl 5,5'-dehydrodivanillate, a model 5-5 lignin-related dimer
- Identified important genes in  $\beta$ -aryl ether catabolic pathways, which are responsible for catabolism of  $\beta$ -O-4 type lignin dimers
- Identified uncharacterized genes in  $\beta$ -1 dimer catabolism, which was enriched in lignin streams
- Demonstrated the functional role of a “three-gene operon” from SYK-6 to improve acetate tolerance in *P. putida*

## Significance

- The findings from this study provide targets for future metabolic engineering in SYK-6 or other microorganisms for the biological valorization of heterogenous lignin monomers and dimers.



Workflow for library assembly and RB-TnSeq: Map the location of barcoded transposons within a host genome; then calculate gene fitness value by enumerating PCR-amplified barcodes after and before a desired selection using BarSeq.



Improved growth of ACB283, *P. putida* overexpressing “three-gene operon” (*otsB*:*SLG\_04500*:*otsA*) at high concentration of acetic acid (125, 150 mM) compared to that with KT2440 (wt).

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