RB-TnSeq Used to Identify Novel Bacterial Tolerance Mechanisms

Background

- Random barcoded transposon insertion sequencing (RB-TnSeq) is a genome-scale method that informs gene essentiality in response to a selective pressure. Once created, an RB-TNSeq library of pooled microbes is a tool which can be used in multiple experiments
- RB-TnSeq was previously used to identify catabolic pathways in *Pseudomonas putida* KT2440, which is the primary microbe used for bioconversion of lignin-derived aromatic compounds in CBI.

Approach

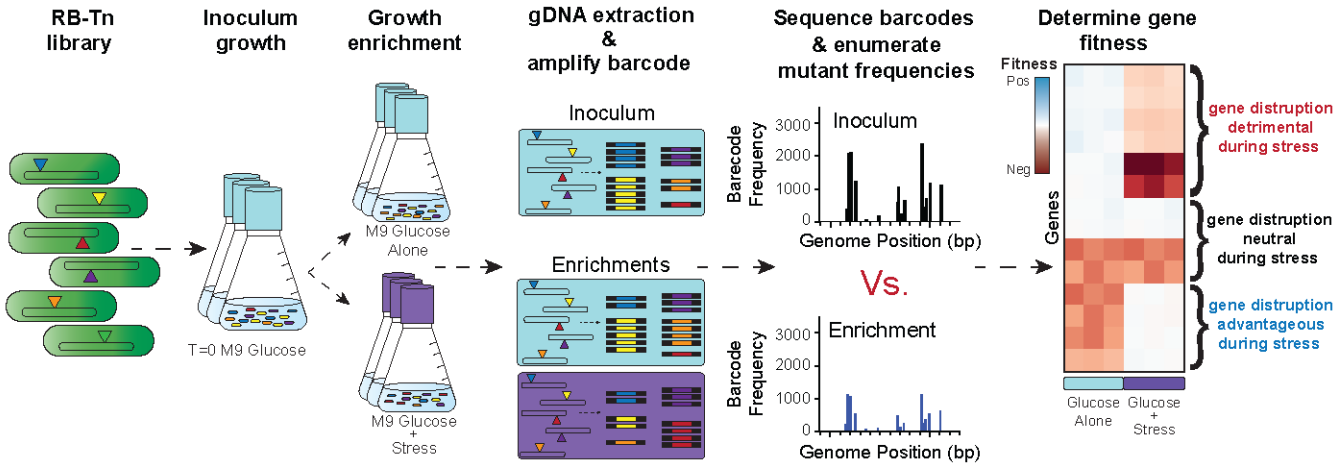
- The RB-TnSeq library in pseudomonas grown in supplemented media to identify tolerance mechanisms to lignin-related aromatic compounds, salt, and carboxylic acids (*right, top*).

Results

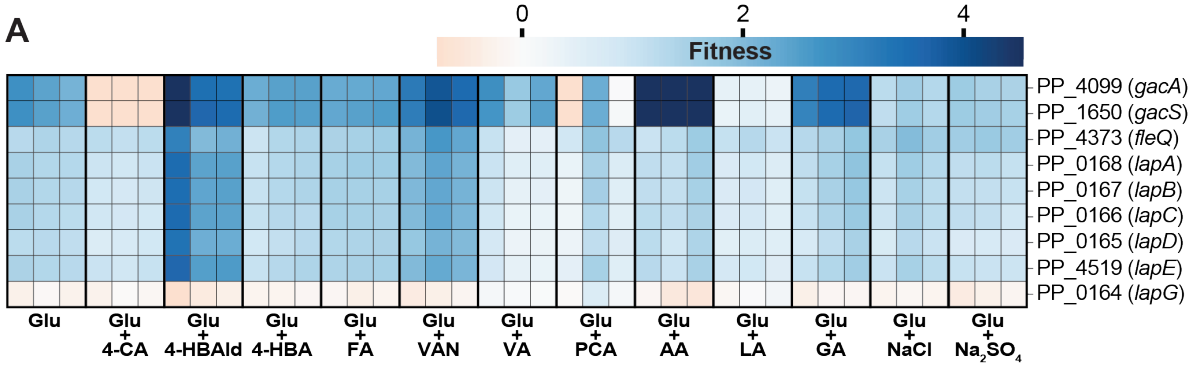
- We found multiple gene hits that were critical for growth in high concentrations of all compounds tested.
- Cell-surface adhesion gene knockouts generally improve growth outcomes in many conditions (*right, bottom*).
- A large suite of non-intuitive gene targets was identified for tolerance to aromatic compounds, acetic acid, and sodium stress.

Significance

- This work elucidates new tolerance mechanisms in *P. putida* KT2440 that can be engineered into production strains used for biological funneling of lignin; this is critical for increased valorization of lignin into other chemicals.



Experimental procedure overview using RB-TnSeq in *P. putida* KT2440



Gene targets from the RB-TnSeq analysis. Heatmap of fitness values for 9 genes involved in the Lap system of cell-surface adhesion. 'Glu' refers to the condition in which the library was passaged from T=0 into a second M9 + glucose culture. Generally, disruption of gacAS, fleQ, or the lapABCDE genes led to improved fitness for all the conditions tested in this work.

Borchert, AJ. *et al*., *Metabol Eng* (2023). doi.org/10.1016/j.ymben.2023.04.007.