Engineering *Pseudomonas putida* for production of muconic acid

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Agile BioFoundry Industry Day
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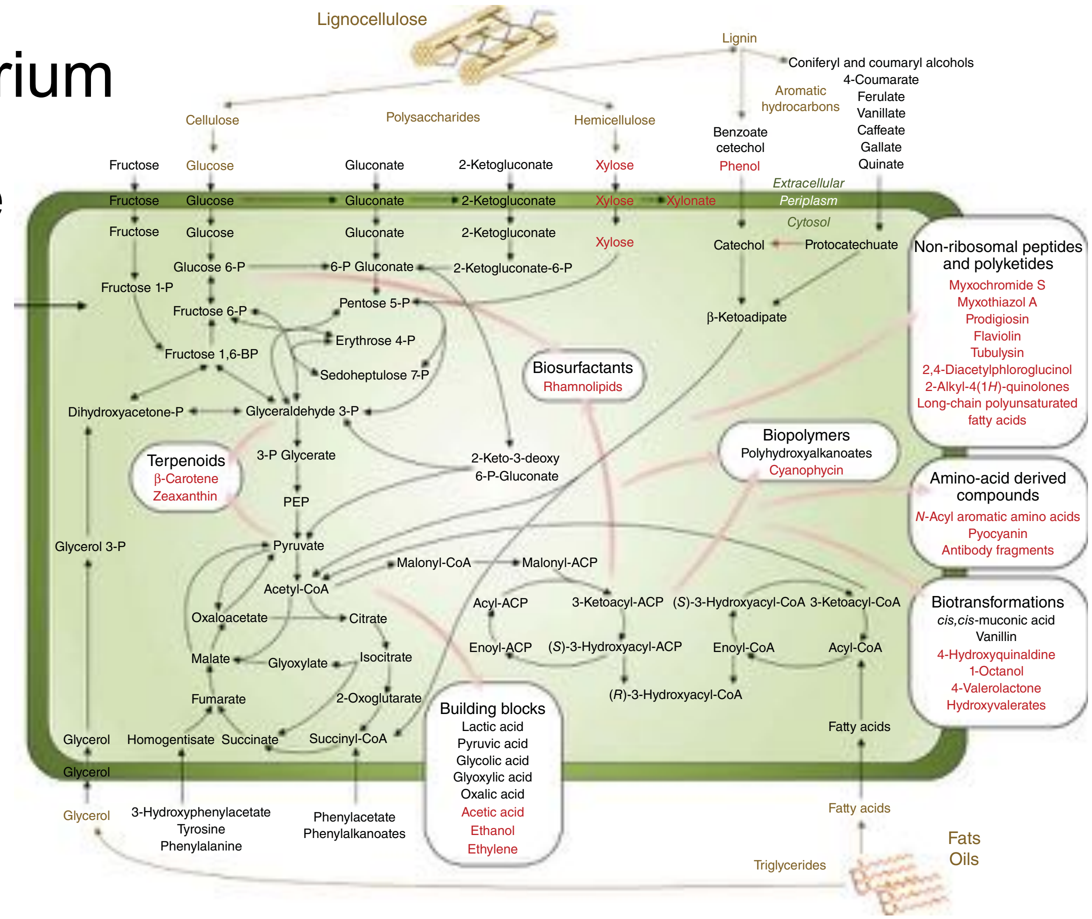
Host: *Pseudomonas putida* KT2440

- Saprophytic soil bacterium
- Gram-negative aerobe
- GRAS
- Fast growing
- Stress tolerant
- Metabolically versatile
- Genetically tractable



Poblete-Castro I, Borrero-de Acuña JM, Nikel PI, Kohlstedt M, Wittmann C. Host Organism: Pseudomonas putida. In: Wittmann C, Liao JC, editors. Industrial Biotechnology: Microorganisms. 2017. p. 337.

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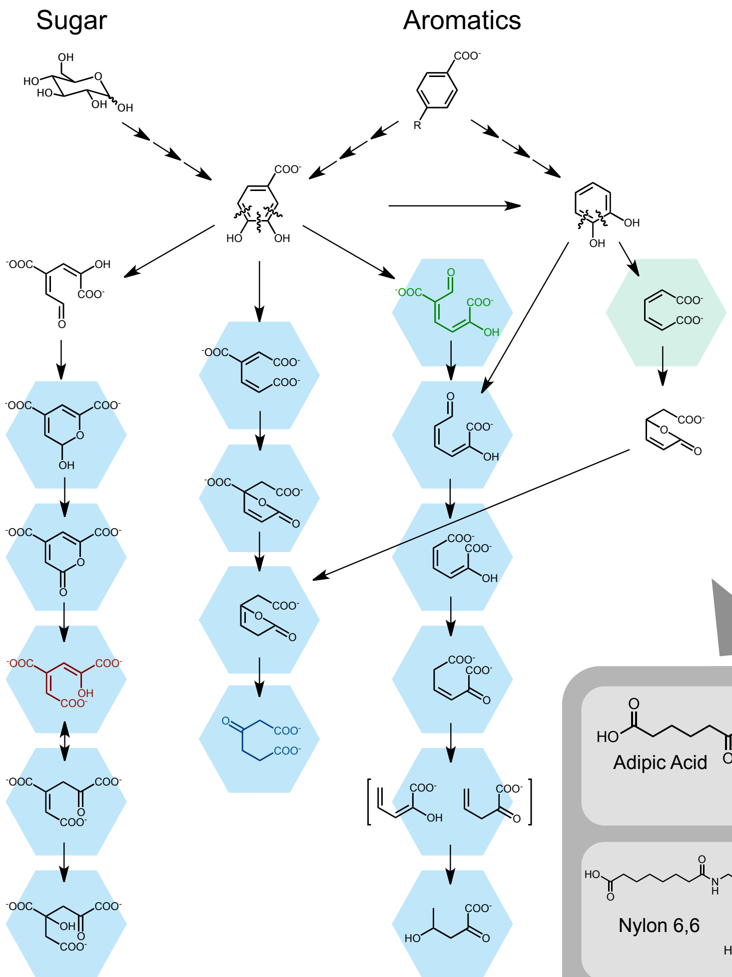
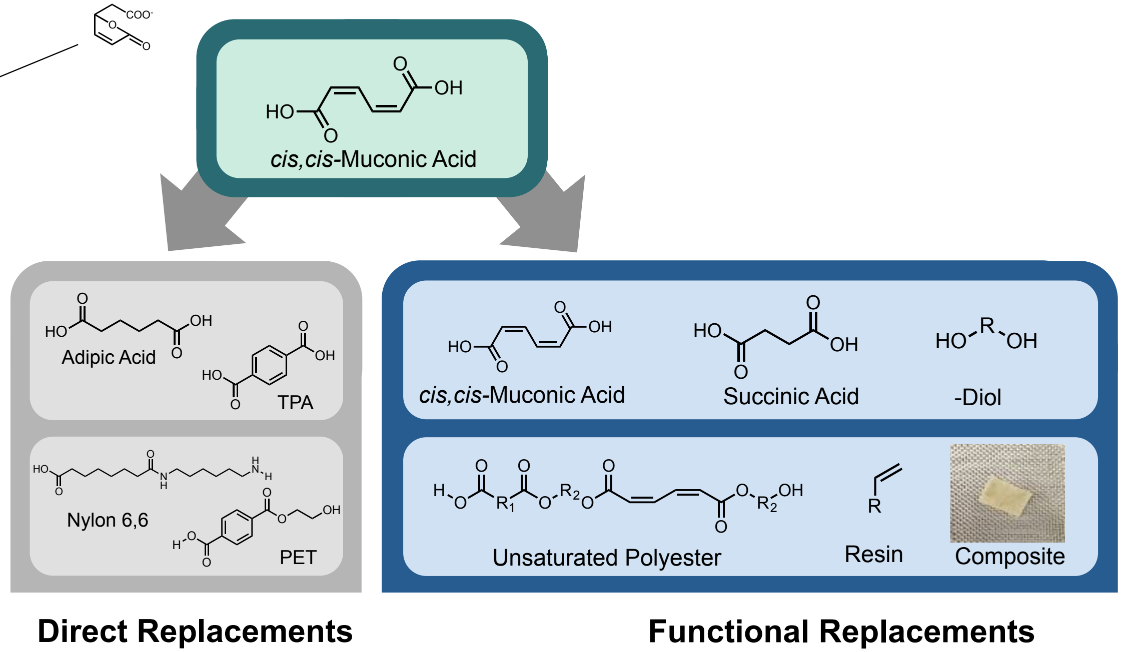
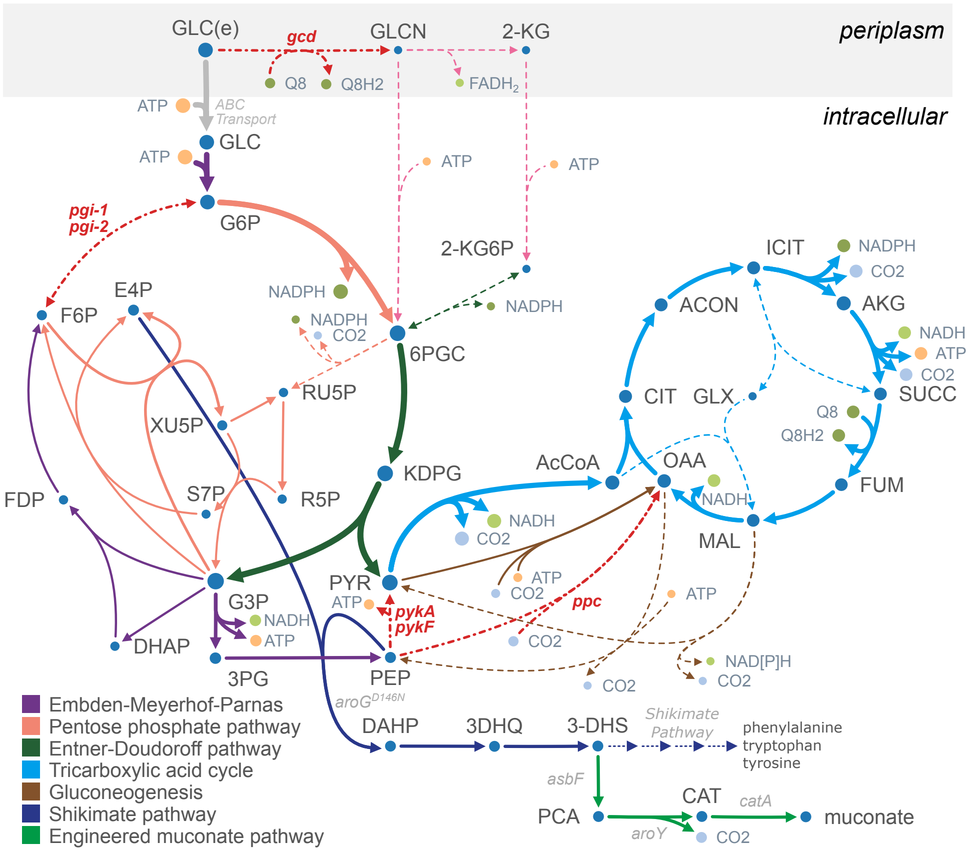
Target: Muconic acid

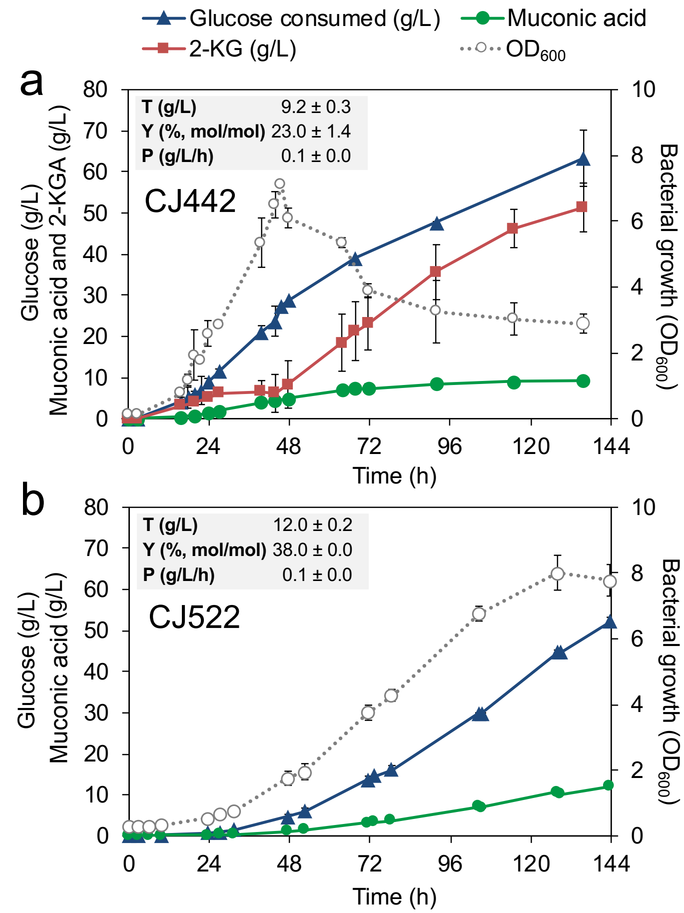


- Aromatic catabolic intermediate
- Unsaturated C6 dicarboxylic acid
- Not readily generated from petrochemicals
- Olefinic double bonds enable additional chemistries (crosslinking, Diels-Alder)



Production of muconic acid from sugar

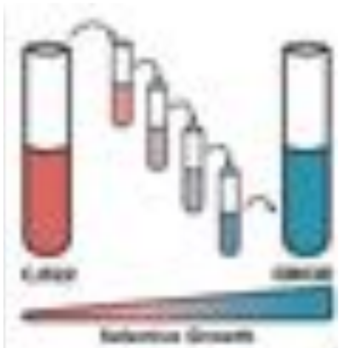


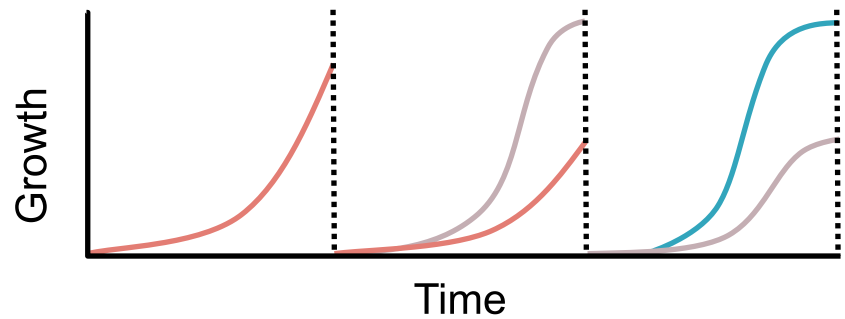


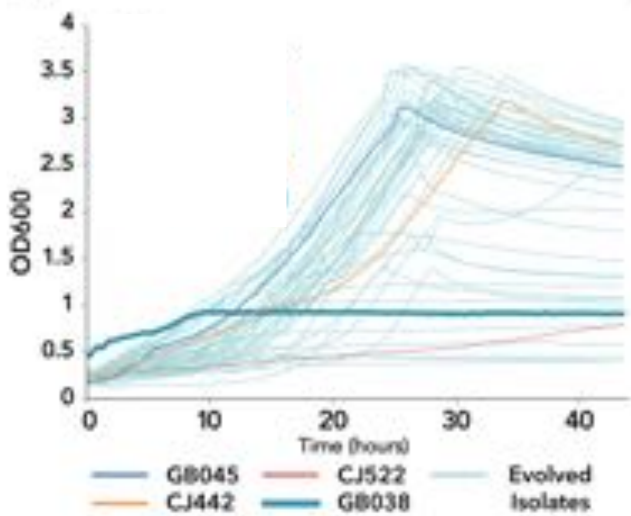
Johnson, C.W., et al., 2019. Innovative Chemicals and Materials from Bacterial Aromatic Catabolic Pathways. Joule 3, 1523–1537.

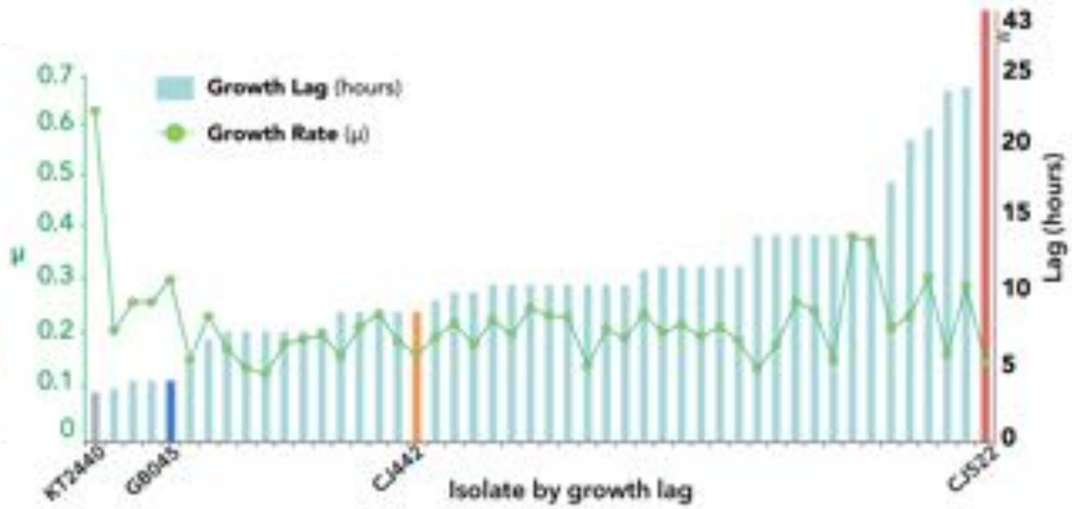
- Engineering achieved ~35% yield of muconic acid from glucose
- Elimination of 2-ketogluconate byproduct slowed growth and productivity

Laboratory evolution to improve growth









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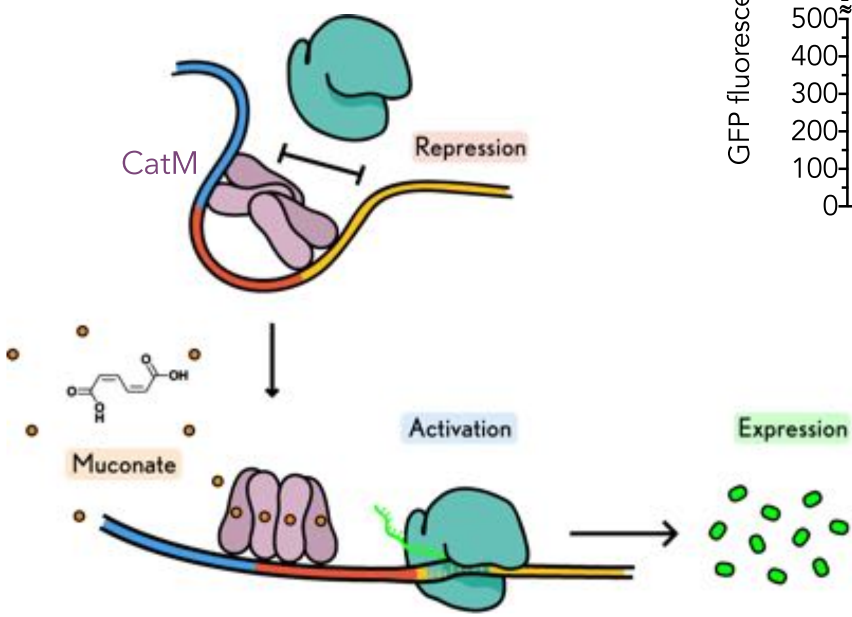
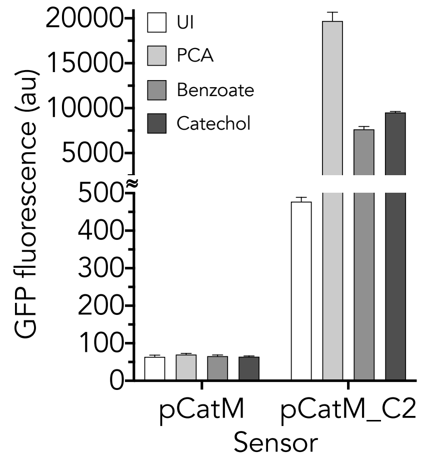
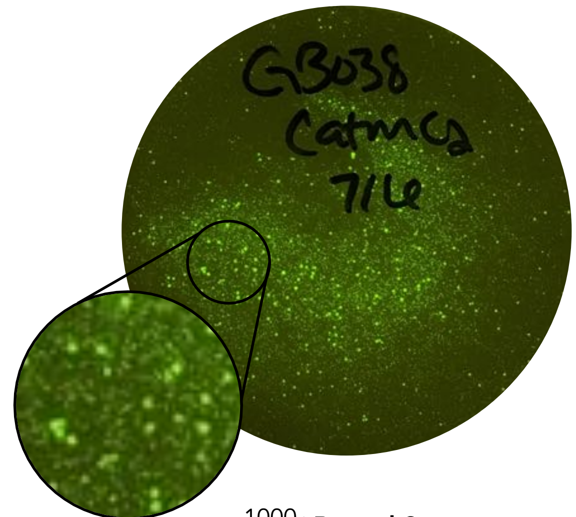
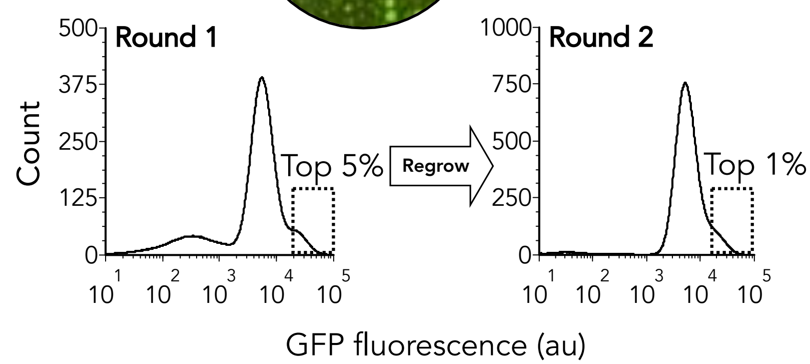
Biosensor development and selection









Adaptive evolution → Muconate Biosensor → Improved production

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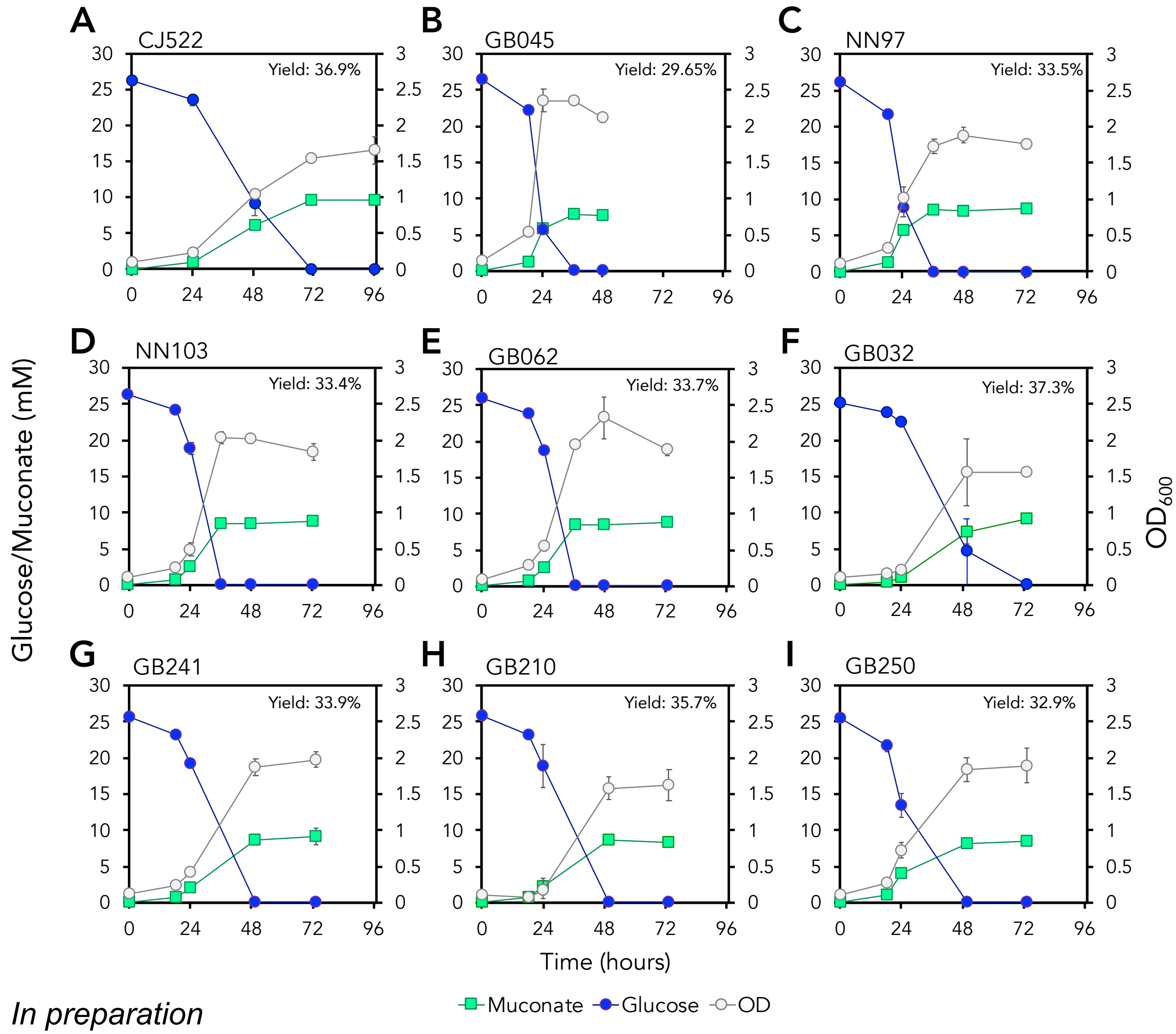
Evolution, screening, and rational engineering improved muconate production



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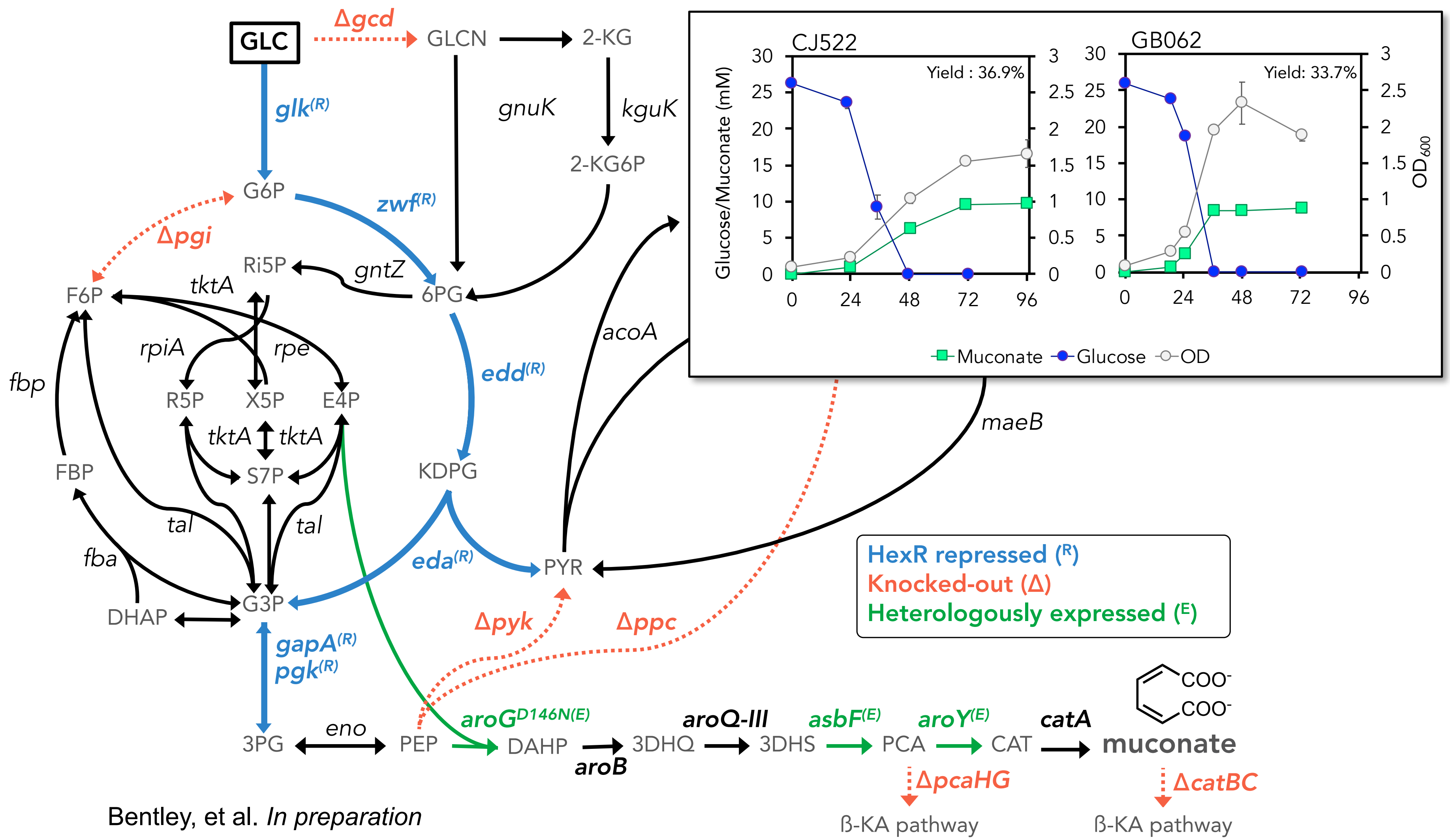
Deletion of *hexR* improves production



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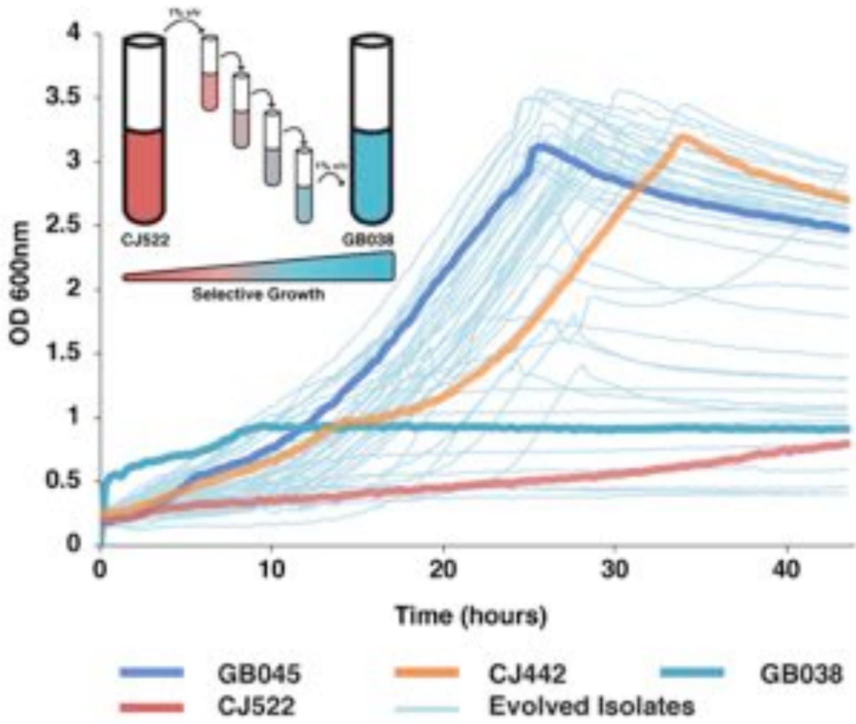
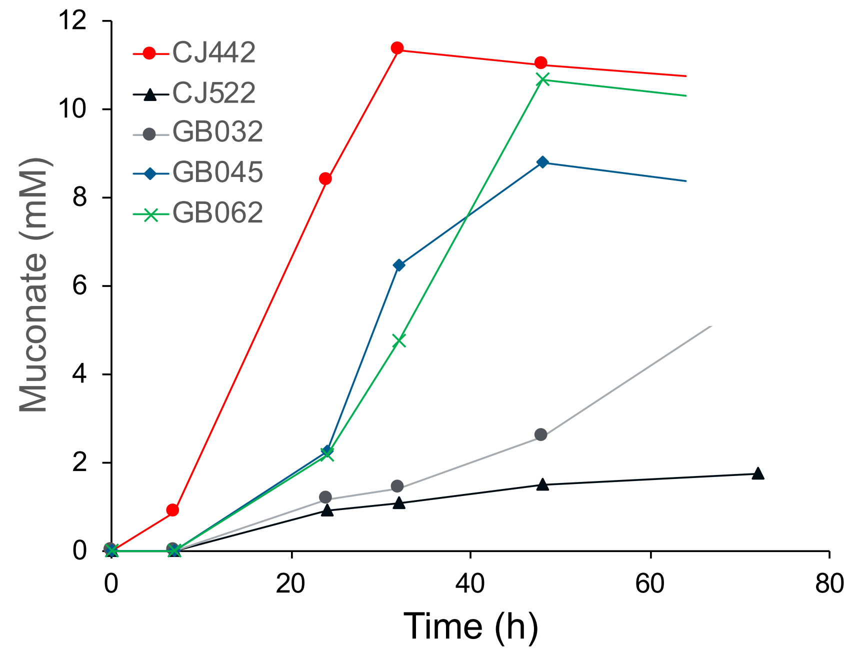
Diverse phenotypes to feed machine learning interface





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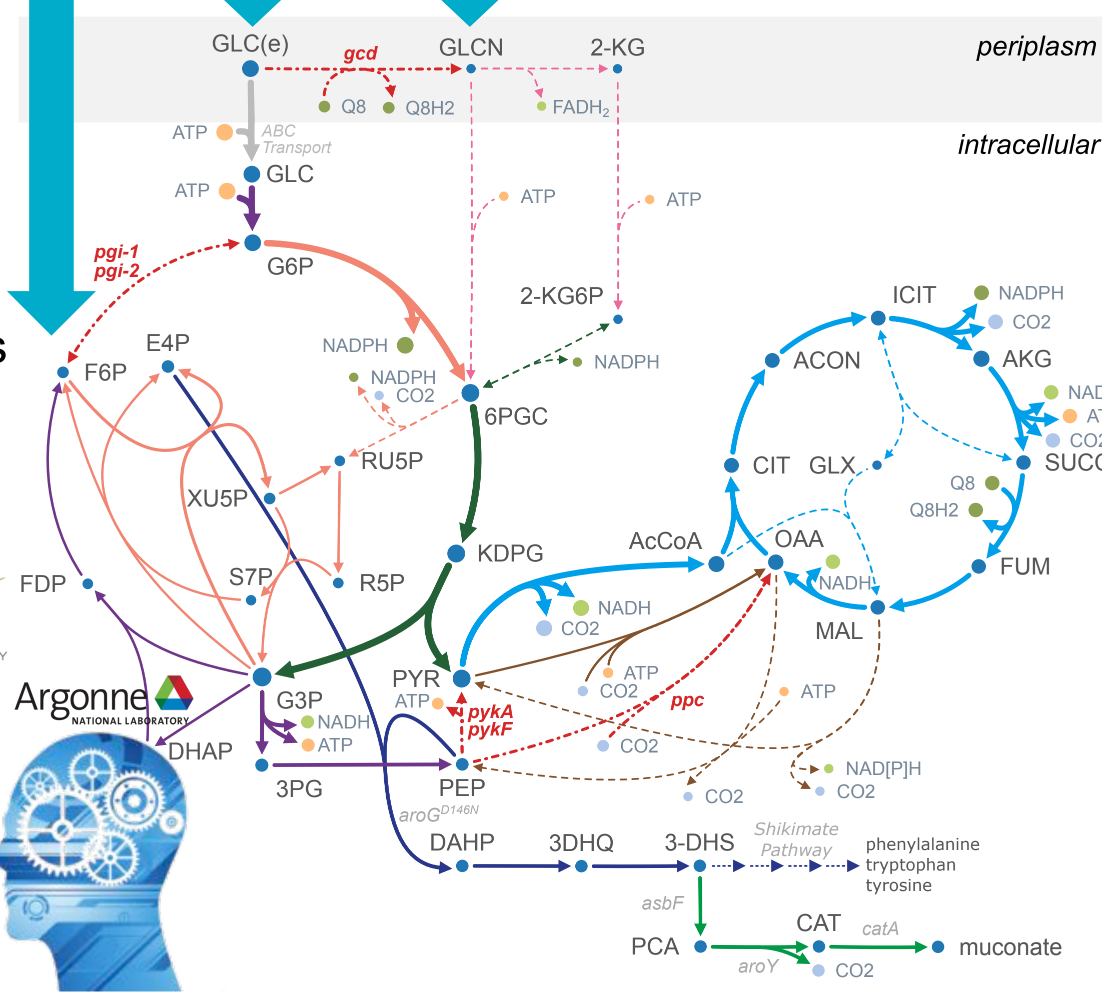
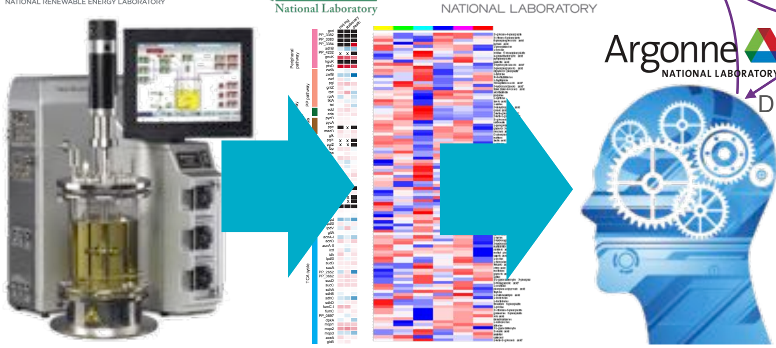
Diverse phenotypes to feed machine learning interface

Input:
- 5 strains with varying muconate and growth phenotypes
- 6 carbon source combinations to query different metabolic nodes

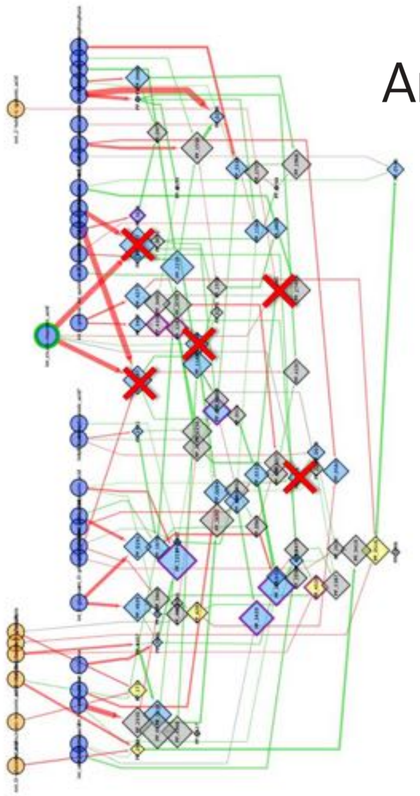
Transcriptomics
Metabolomics
Proteomics

Fructose **Glucose** **Gluconate**





Going beyond rational engineering with machine learning



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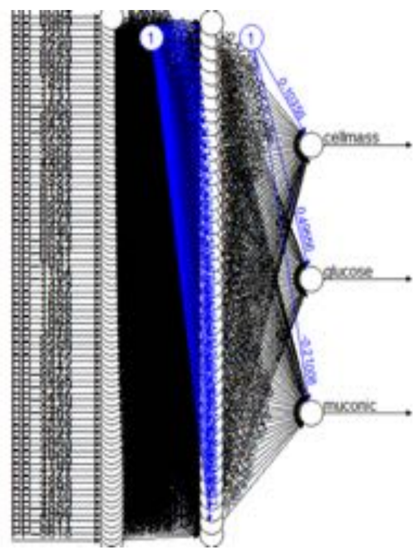


Predict TF expression pattern as functions of (observed) metabolome and (modeled) TF KO

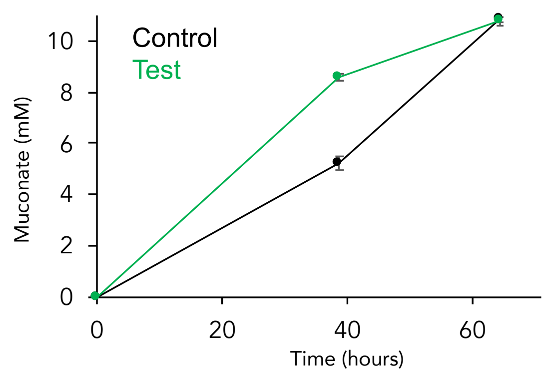
Use predicted TF expression pattern as input to ANN

Predict fermentation phenotype, relative to strain without TF knockout

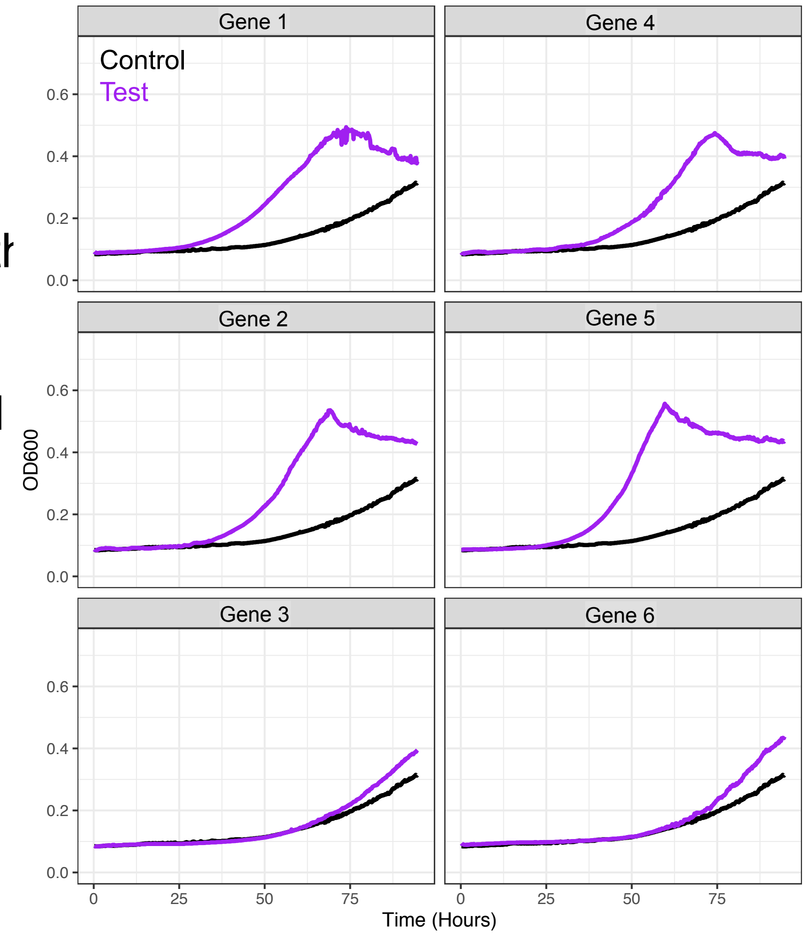
Evaluating non-intuitive Learn targets

- Machine learning targets were provided on August 1, 2019
- Constructs to either delete or overexpress genes were constructed
- Resulting strains were generated, all witl chromosomal modifications
- By September 30, 2019, 111 strains had been generated AND characterized for performance, in biological triplicate

Improved productivity



Improved growth



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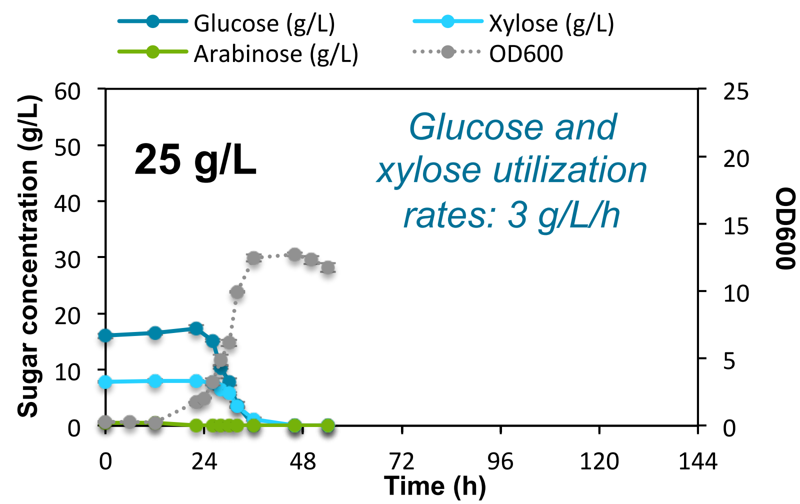
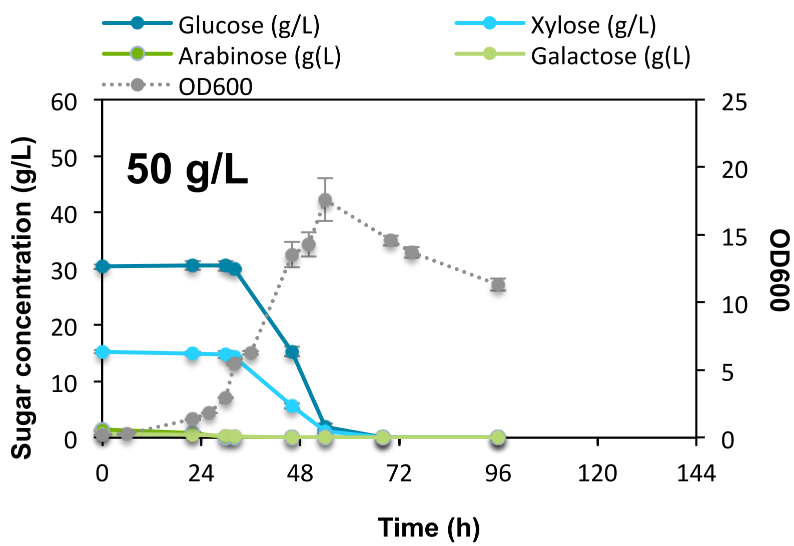
Enabling Hydrolysate Utilization in *P. putida*

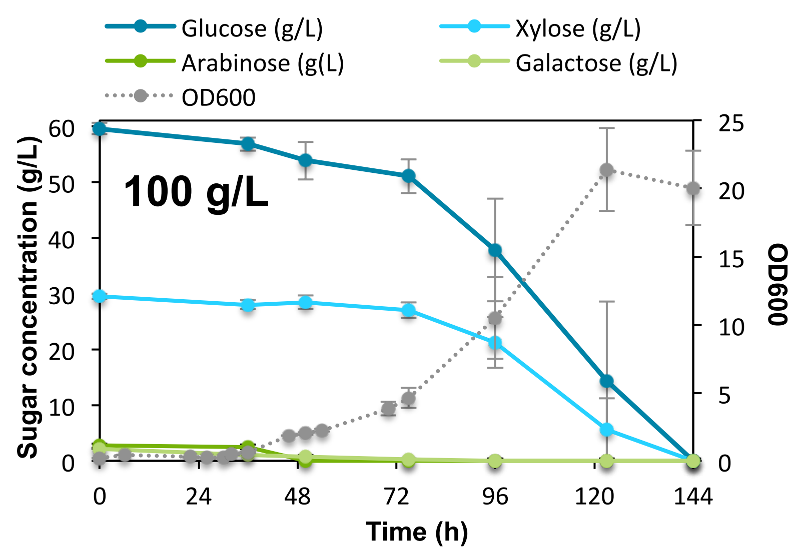
Oak Ridge National Laboratory

Corn Stover DMR-EH
(deacetylation and mechanical refining, enzymatic hydrolysis)

Glucose	85 g/L (472 mM)
Xylose	37 g/L (245 mM)
L-arabinose	5.5 g/L (37 mM)
Galactose	1.2 g/L (7 mM)







Acknowledgements

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