

# Optimal production of *Phanerochaete chrysosporium* manganese peroxidases and *Trametes* sp. C30 laccase hybrid Lac131 in *Aspergillus niger* for lignin bioconversion

## Background/Objective

- Incorporating the production of ligninolytic enzymes into industrial filamentous fungus *A. niger* will enhance the bioconversion of lignocellulosic biomass to various biofuels and bioproducts

## Approach

- Transgenic expression of *P. chrysosporium* manganese peroxidases (mnPs) and *Trametes* sp. C30 laccase hybrid Lac131 were optimized in *A. niger*

## Results

- The supplementation of bovine hemoglobin or soy proteins, and deletion of *prtT* and *vsm1* regulators significantly improved mnP production in *A. niger*
- Both mnP and laccase decolorized dyes and cleaved model lignin dimers

## Significance/Impacts

- Successfully produced ligninolytic enzymes including mnP and laccase in *A. niger* by optimizing the culture conditions and host strain
- Co-production of mnP and laccase in engineered *A. niger* will enhance the lignin bioconversion to value-added chemicals

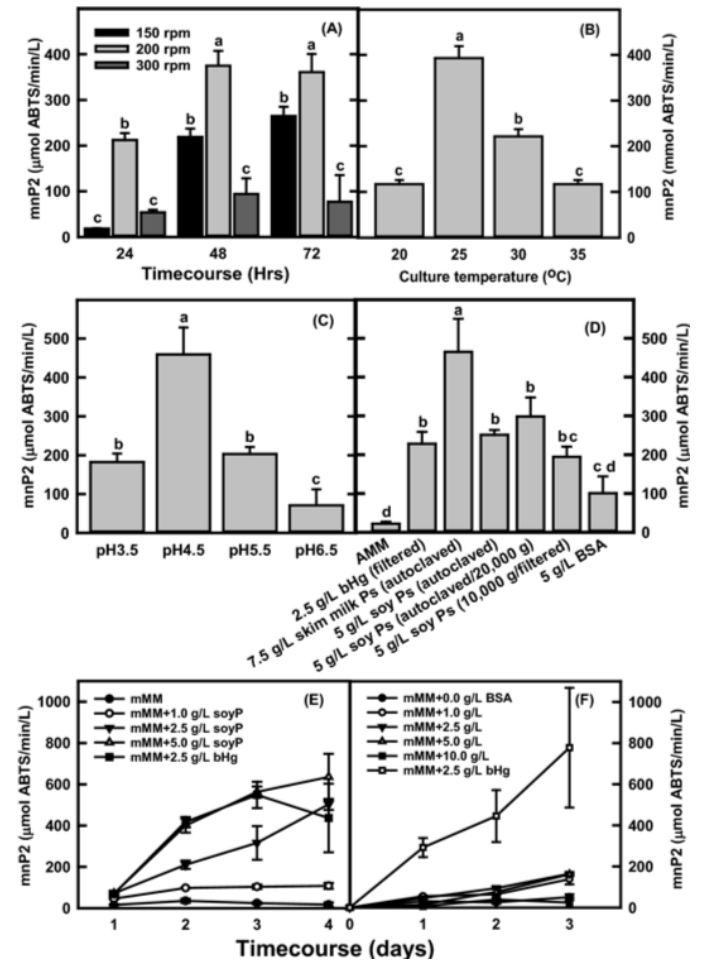


Figure 1: Optimization of culture conditions for manganese peroxidase (mnP) production in *Aspergillus niger*. The effects of (A) agitation, (B) temperature, (C) initial pH, and (D)-(F) supplementation with bovine hemoglobin (bHG), skim milk, and soy proteins (soy P) on the mnP production were investigated.

# Heteropoly acid catalysts for limonene oxide isomerization: the correlation of catalyst acid strength to activity and selectivity

## Background/Objective

- Limonene is target biological compound that can be converted to many value-added molecules
- Previous work examined efficient oxidation to limonene oxide
- Need to explore efficient limonene oxide isomerization to dihydrocarvone

## Approach

- Explore the relationship between catalyst acid strength and the activity and selectivity for the isomerization reaction

## Results

- Experimental testing of a broad range of solid acid catalysts showed a direct correlation between acid strength and activity and selectivity. Improved method for acid strength measurement also developed

## Significance/Impacts

- High selectivity to the desired dihydrocarvone product could be achieved with proper selection of the solid acid catalyst

Lee, H., et.al. *Catalysis Science and Technology*. doi: 10.1039/D5CY00747J (JBEI #1268)

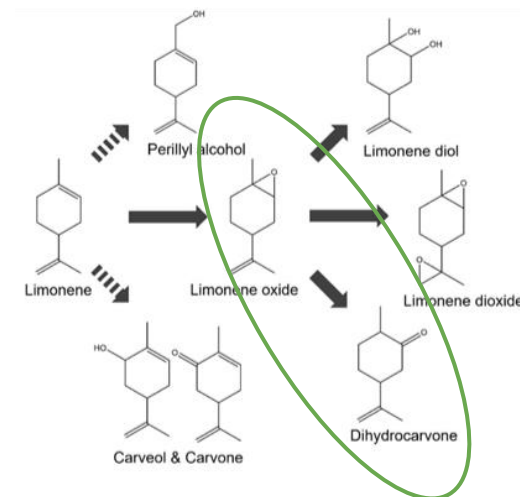


Figure (A): Limonene oxide isomerization to dihydrocarvone.

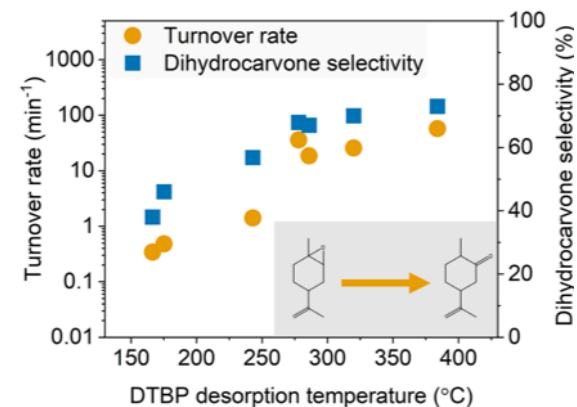


Figure (B): Increasing acid strength increased both reaction activity and selectivity.

# Elucidating the Competitive Hydrodeoxygenation of Lignin-Derived Oxygenates over Bulk MoO<sub>3</sub> Catalyst through Kinetic Analysis

## Background/Objective

- MoO<sub>3</sub> has been found to be effective for the HDO of lignin derivatives giving aromatics, alkenes and alkanes
- Previous kinetic studies used single model compounds
- Exploring the impact of competitive adsorption on the HDO reaction products

## Approach

- Perform extensive experimental kinetic analysis of binary lignin-surrogate molecules to understand adsorption/reaction behavior

## Results

- 3 distinct acid sites were found on MoO<sub>3</sub>; 2 Brønsted type and one Lewis type (key HDO site)
- HDO reaction mechanism does not change as the number of oxygenates in the system increases

## Significance/Impacts

- Greater insight developed into the simultaneous HDO of complex lignin deconstruction product mixtures to aromatics, alkenes and alkanes

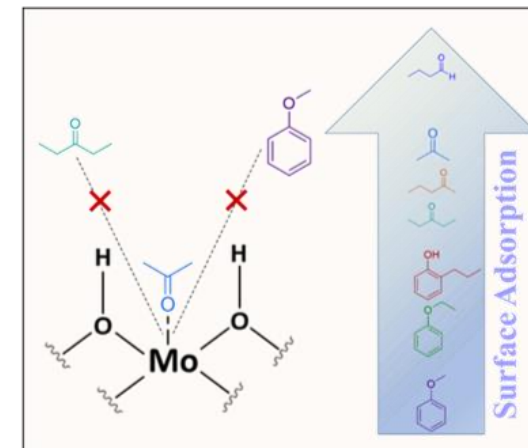


Figure A: Competitive adsorption of binary mixtures of lignin derivatives showed the relative absorptivity of the molecules.

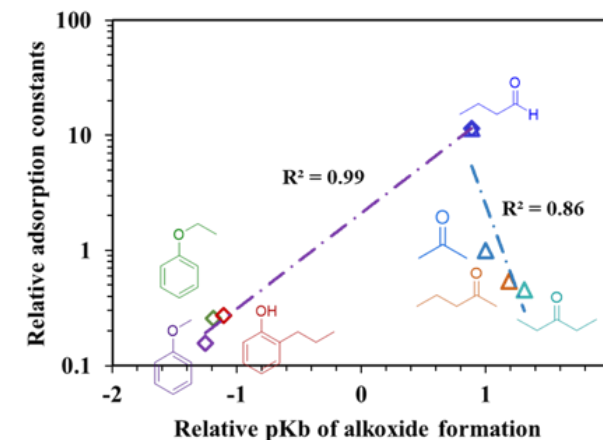


Figure B: Relationship between adsorption constant relative to acetone and pK<sub>b</sub> value of alkoxide formation relative to acetone for different oxygenates.

Khan, M., et.al. *ACS Catalysis*. doi: 10.1021/acscatal.5c04264 (JBEI #1269)

# Metabolic engineering and synthetic biology for the environment: from perspectives of biodetection, bioremediation, and biomanufacturing

## Background

- Chemical synthesis enables large-scale production and technological advancement; rapid industrialization causes widespread pollution threatening health and ecosystems
- Metabolic engineering and synthetic biology approaches have been developed to enable pollutant biosensing, bioremediation, and biomanufacturing

## Reviewed contents

- Biosensor development for pollutant monitoring
- Microbial bioremediation for environmental restoration
- Engineering of microorganisms to degrade and convert diverse feedstocks, including agriculture residues and waste streams,
- Biosynthesis of valuable fuels, materials, and natural products

## Conclusion and future perspectives

- Engineering microbes enable pollutant detection, cleanup, and sustainable production
- Advancing biosensors expand monitoring through omics and synthetic biology tools
- Optimizing strains and pathways boost bioremediation and biomanufacturing efficiency

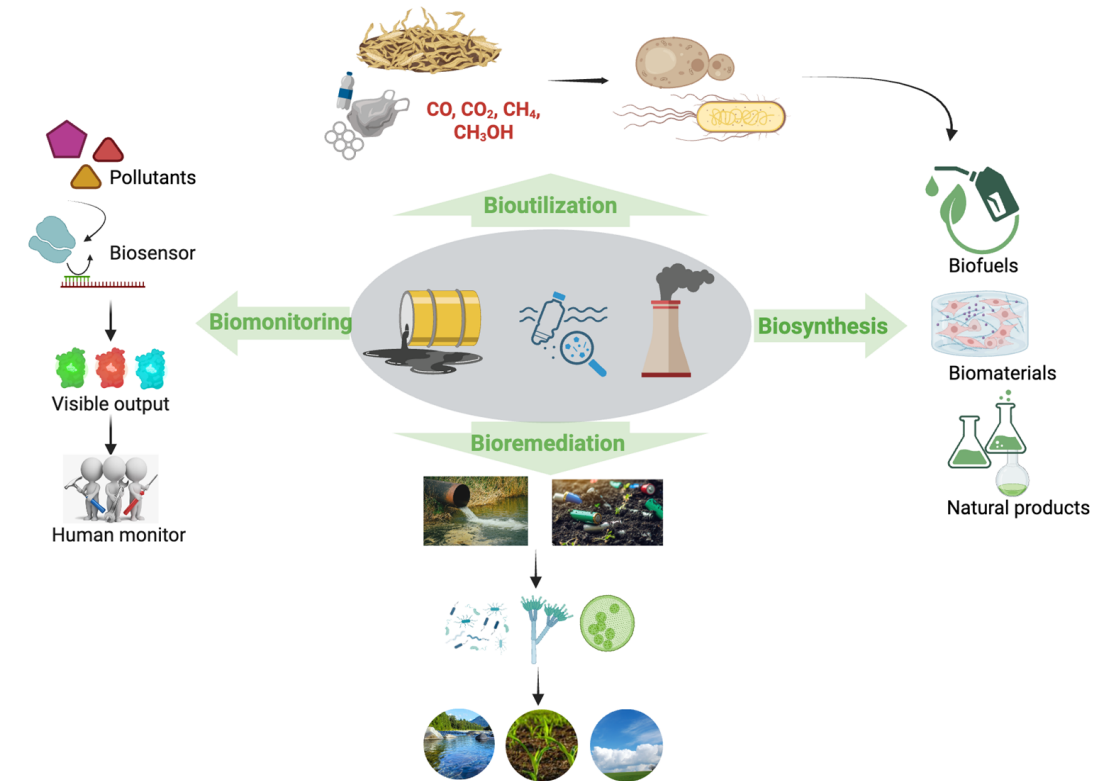


Figure A: Metabolic engineering and synthetic biology approaches for environmental improvement, including biomonitoring, bioremediation, and biomanufacturing.

# Automated strain construction for biosynthetic pathway screening in yeast

## Background/Objective

- Automation accelerates the DBTL cycle; however, yeast engineering remains low-throughput
- Objective: program a modular and integrated robotic pipeline to streamline strain construction

## Approach

- We programmed the robot Hamilton VANTAGE to perform transformation of yeast (*S. cerevisiae*)

## Results

- Our pipeline delivers **2,000 strains/week**
- As POC, it improved titers by **5X** in one round by screening 300 candidates in the biosynthesis of verazine (intermediate of a anti-cancer drug)

## Significance/Impacts

- Increasing throughput at JBEI to thousands of strains will enable scientists to deliver and scale bioproducts faster

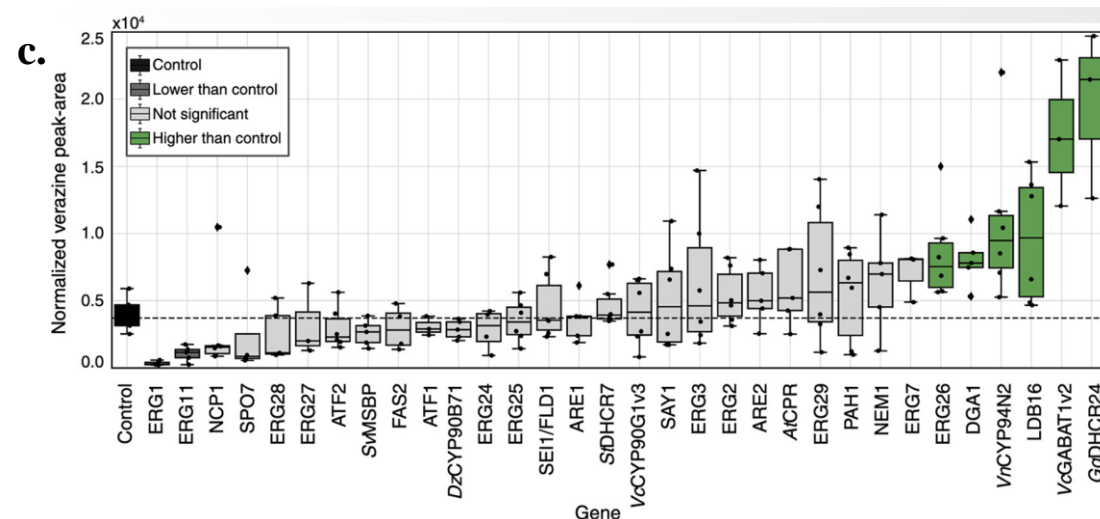
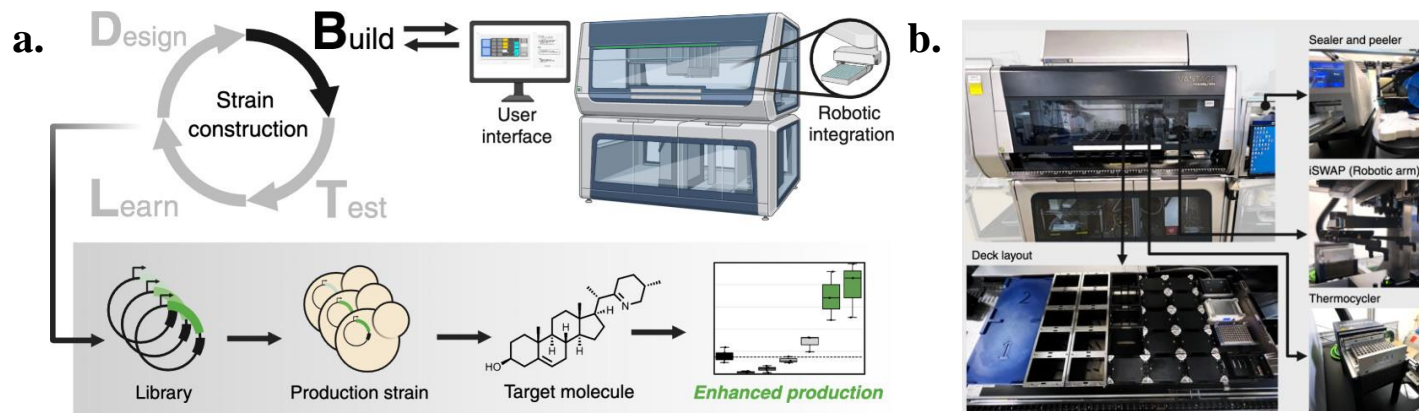


Figure caption: **Automating strain construction of yeast.** a) Overview of the pipeline for building *S. cerevisiae*. b) The Hamilton VANTAGE and how we leveraged its central robotic arm to integrate multiple hardware c. Results of screening hundreds of samples to increase biosynthesis of the target molecule verazine.

# Engineering 2-Pyrone-4,6-Dicarboxylic Acid Production Reveals Metabolic Plasticity of Poplar

## Background/Objective

- Generating bioproducts directly in plants can enhance the overall value of biomass, contributing to more cost-effective biorefinery operations
- This work aims to engineer poplar (*Populus* spp.) to produce 2-pyrone-4,6-dicarboxylic acid (PDC), a versatile chemical precursor for the synthesis of bioplastics and other high-performance materials

## Approach

- An artificial pathway composed of five microbial genes enabling *de novo* PDC production was introduced and expressed in poplar stems

## Results

- Transgenic poplars produced PDC along with elevated levels of the biosynthetic intermediate protocatechuic acid and its derivative vanillic acid
- These plants exhibited markedly reduced lignin deposition and increased hemicellulose content, resulting in enhanced saccharification efficiency
- Additionally, transgenic roots and bark exhibited enhanced suberization, conferring greater tolerance to salt stress

## Significance/Impacts

- The study demonstrates the potential of poplar as a host for the cost-effective production of high-value bioproducts

Dwivedi, N., et.al. *Plant Biotechnology Journal*. doi: 10.1111/pbi.70414 (JBEI #1272)

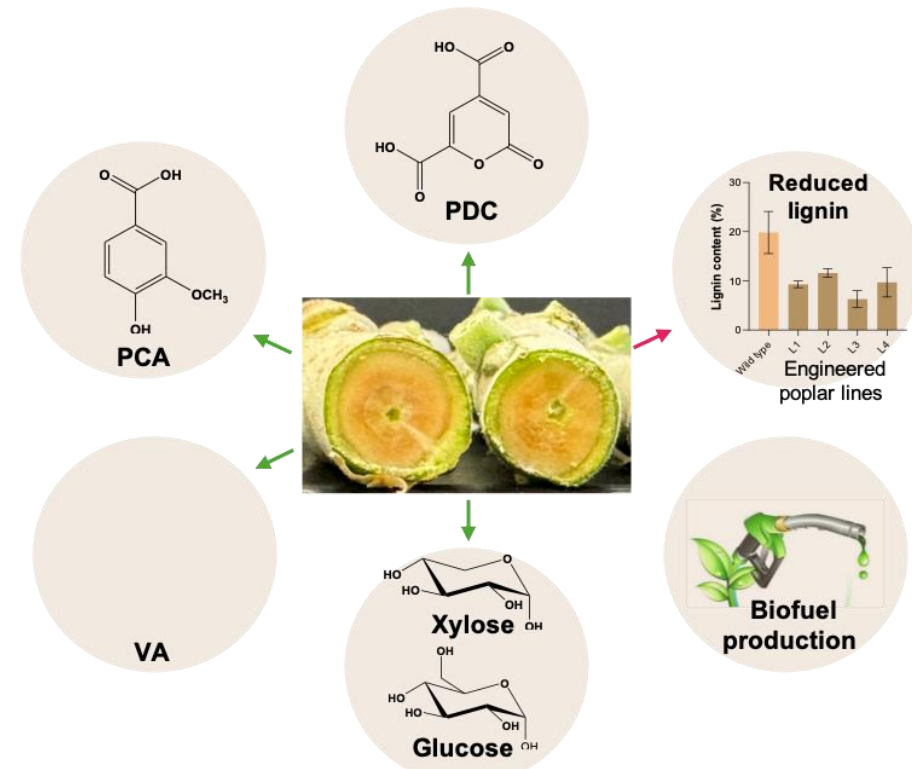


Figure caption: Engineered poplar, shown in the cross-section photo, can produce high levels of valuable bioproducts. These include: 2-pyrone-4,6-dicarboxylic acid (PDC), which can be used to make degradable plastics, and protocatechuic acid (PCA) and vanillic acid (VA), which both have industrial and pharmaceutical applications. The engineered poplar also had reduced cell wall lignin content. This enhances the release of simple sugars such as xylose and glucose that can be refined into biofuels and other bioproducts.

# Feedstock-efficient conversion through hydrogen and formate-driven metabolism in *Escherichia coli*

## Background/Objective

- Use H<sub>2</sub> or formate supplementation to decrease variable feedstock costs and increase efficiency in *E. coli*

## Approach

- Expressed Soluble Hydrogenase from *C. necator* H16 or FDH from *Pseudomonas* sp. 101 in *E. coli*
- Monitored feedstock usage, H<sub>2</sub>/formate, and metabolites to examine efficiency using Flux Balance Analysis (FBA)

## Results

- 13-gene hydrogenase operon functionally expressed on a single plasmid
- 86.6% of electrons from H<sub>2</sub> and 98.4% from formate replaced internal feedstock oxidation
- 57.6% boost in mevalonate titers (a precursor for biofuels and pharmaceuticals)

## Significance/Impacts

- Foundational step towards feedstock-efficient conversion—using renewable electricity for biomanufacturing instead of relying on sugar or petrochemical inputs

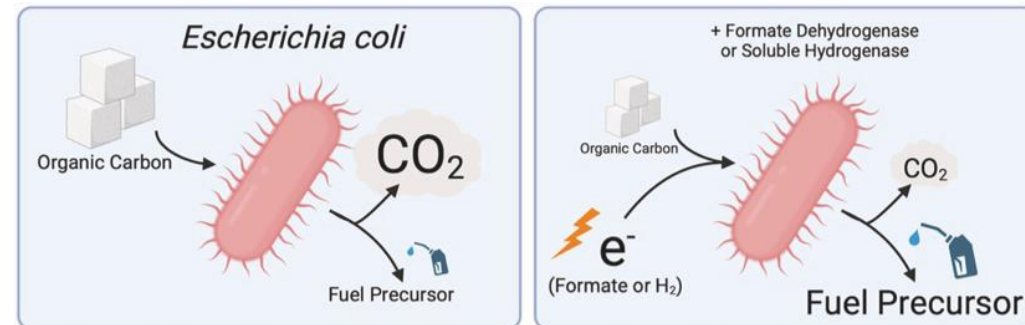


Figure caption (A): Graphical abstract

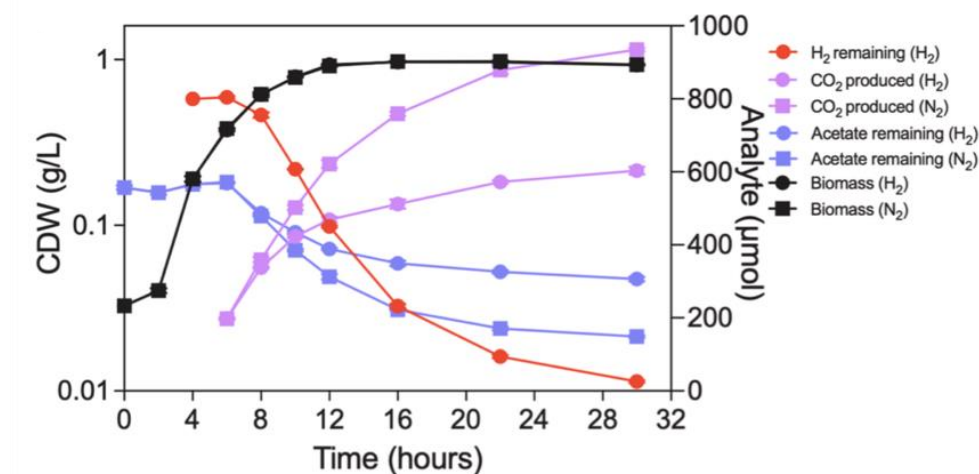


Figure caption (B): Growth curve of *E. coli* expressing H<sub>2</sub>ase, measuring suppression of feedstock (acetate) oxidation and CO<sub>2</sub> evolution when treated with H<sub>2</sub> (relative to N<sub>2</sub> treatment)

# Biosensor-driven strain engineering reveals key cellular processes for maximizing isoprenol production in *Pseudomonas putida*

## Background/Objective

- Synthetic biology generates vast combinatorial designs, yet high-throughput analytical methods to screen them are poorly matched to interrogate this genomics-era search space

## Approach

- We developed end-to-end modular workflows to demonstrate a biosensor-driven, growth-coupled selection strategy in *Pseudomonas putida* tailored to isoprenol, a potential aviation fuel precursor

## Results

- Iterative combinatorial strain engineering derived from these hits yielded a 36-fold titer increase to ~900 mg/L. Integrated -omics analysis revealed that metabolic rewiring towards amino acid catabolism was crucial for this improvement
- Amino acid supplementation was found to be beneficial by techno-economic analysis

## Significance/Impacts

- Our modular workflow provides a powerful strategy for optimizing complex heterologous pathways and uncovering emergent host biology
- We demonstrate an antibiotic-free platform strain for isoprenol biomanufacturing

Menasalvas, J., et.al. *Science Advances*. doi: 10.1126/sciadv.ady2677(JBEI #1274)

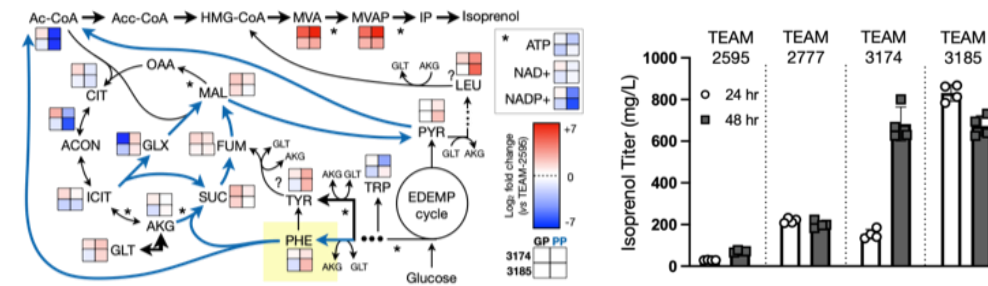
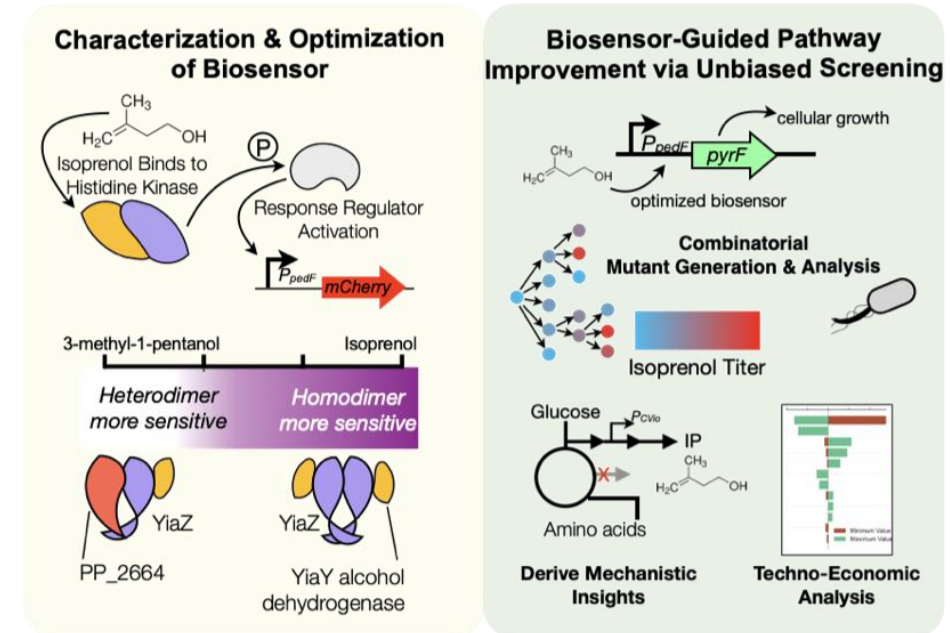


Figure caption: Top: Graphical abstract of work. Bottom left: Central metabolic map of rewired strains showing relative fold changes of metabolic pools. Bottom right: Isoprenol titer in engineered *P. putida*

# The mevalonate pathway of isoprenoid biosynthesis supports metabolic flexibility in *Mycobacterium marinum*

## Background/Objective

- *Mycobacterium marinum* (Mm) encodes both the methylerythritol phosphate (MEP) and the mevalonate (MEV) isoprenoid synthesis pathways
- Here, we aim to understand the role of the MEV pathway in Mm and its impact on metabolic flexibility

## Approach

- A  $\Delta$ MEV mutant (deletion of the MEV pathway) was created to determine pathway essentiality, while bioinformatics, gene editing, and metabolomic analyses were employed to study Mm isoprenoid synthesis pathways
- MEV pathway expression under environmental stress was also studied

## Results

- $\Delta$ MEV mutant grew normally confirming its nonessential status
- MEV pathway modulation shows compensatory changes in MEP intermediates
- Upregulation of the MEV pathway during hypoxic conditions suggests a role in metabolic adaptation

## Significance/Impacts

- The MEP pathway is essential in Mm whereas the MEV pathway is dispensable in culture but may confer metabolic flexibility for environmental adaptation

Qabar, C., et al. J Bacteriol. 10.1128/jb.00287-25 (JBEI #1275)

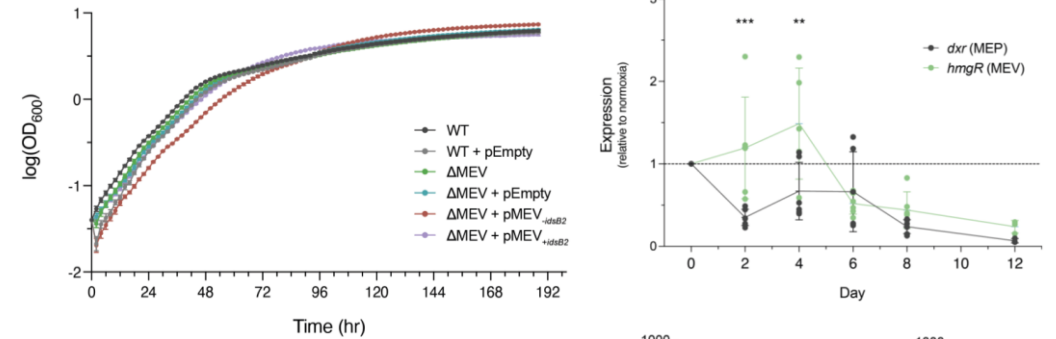


Figure 1: The MEV pathway is nonessential in Mm.

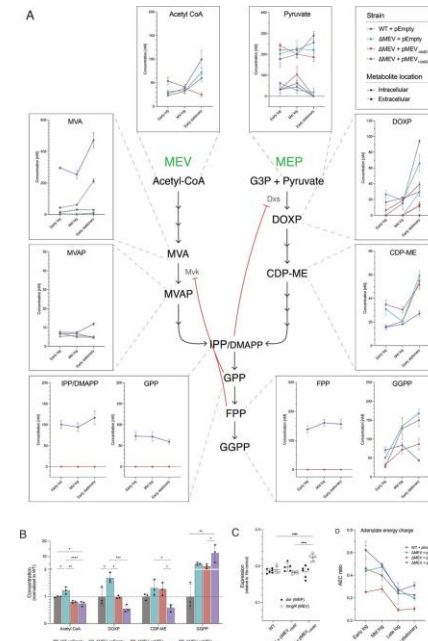


Figure 2: Modulating either isoprenoid biosynthesis pathway impacts metabolism in the other.

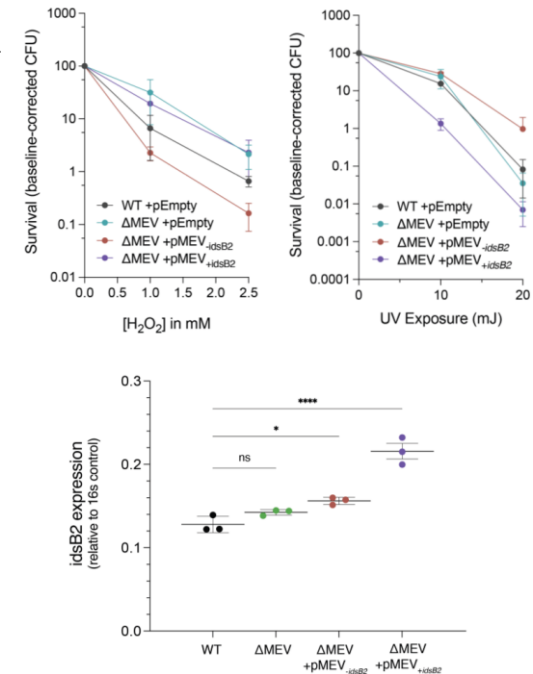


Figure 3: MEV pathway expression has functional consequences under environmental stresses.

# Enabled Publications

# Production of triacetic acid lactone from oleic acid by engineering the yeast *Candida viswanathii*

## Background/Objective

- Triacetic acid lactone (TAL), a simple polyketide, holds significant potential as a platform chemical for synthesizing various high-value compounds essential in industrial processes.
- Oleaginous yeast, *Candida* strains have demonstrated to be remarkable producers of dicarboxylic acids from alkanes and fatty acids.

## Approach

- Adaptive laboratory evolution (ALE) / Strain engineering for TAL pathway
- Multi-omics characterization of the strains (proteomics and metabolomics)

## Results

- The ALE and engineered strains resulted in up to 50-fold enhancement in TAL titers
- Multi-omics data provided valuable information of overexpressed proteins and metabolites in the TAL pathway

## Significance/Impacts

- This is the first example of the production of TAL from oleic acid using engineered and evolved *Candida viswanathii* strains

Ghosh, S., et.al. *Microb Cell Fact.* doi: 10.1186/s12934-025-02841-7 (JBEI #124)

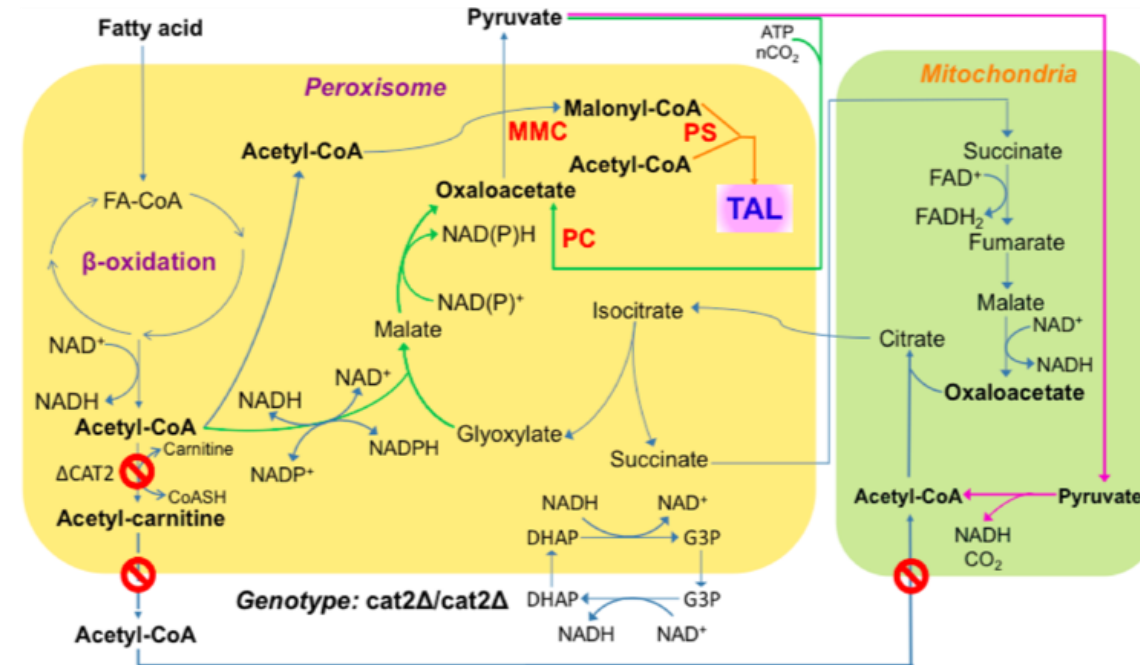


Figure caption: Genetic modifications applied to *C. viswanathii*. Deletion of the CAT2 gene retained the acetyl-CoA pool within the peroxisome. Peroxisomal expression of methylmalonyl-CoA carboxyltransferase (MMC) and pyruvate synthase (PS) enzymes promoted TAL production from acetyl-CoA while pyruvate carboxylase (PC) was expressed to replenish oxaloacetate. The overexpressed enzymes are indicated in red.

# Thermal power plant proximity alters Olive composition and induces cytotoxicity in human cells

## Background/Objective

- Thermal power plants (TPPs) are essential for meeting increasing energy demands, but they also pose significant environmental and health risks
- We investigated the biochemical responses of olives grown at varying distances (close, middle, and distant) from the Yatağan TPP in Türkiye

## Approach

- We analyzed olives to assess differences in phenolic and flavonoid profiles, essential nutrients, toxic metal content, and biological activity

## Results

- Olives grown near the TPP exhibited reduced levels of essential nutrients and elevated concentrations of toxic metals, with their extracts showing cytotoxic effects on normal human cells derived from the breast, retina, vein, and bronchus

## Significance/Impacts

- This study highlights the need for continuous environmental monitoring and regulatory measures to mitigate heavy metal contamination and ensure the sustainability of olive cultivation in regions surrounding TPPs

Gürbüz, E., et.al. *Sci Rep.* doi: 10.1038/s41598-025-18066-y (JBEI #125)

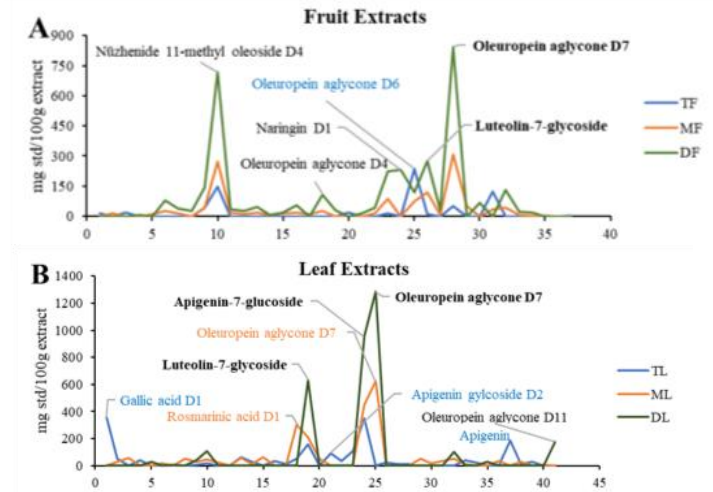


Figure caption 1: Chemical composition of (A) fruit and (B) leaf extracts by region: T (closest), M (middle), D (farthest); F = fruit, L = leaf

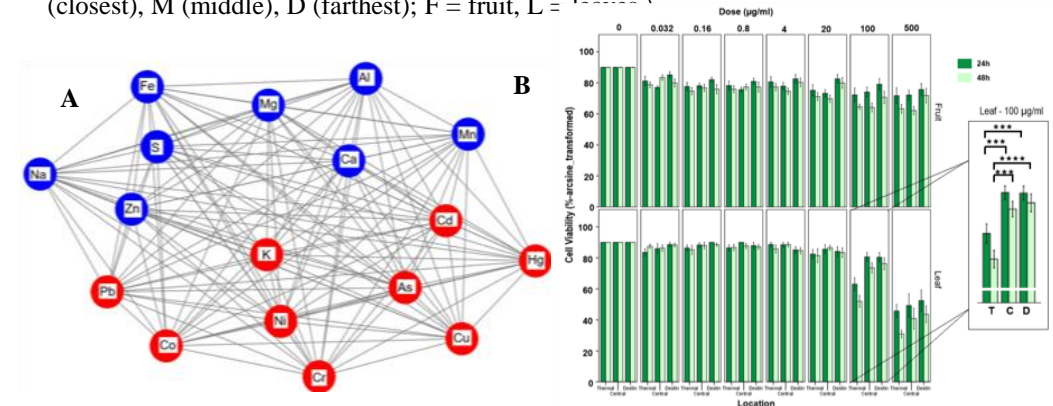


Figure caption 2 : (A) Correlation network of elemental changes in olive trees. Blue nodes represent downregulated essential nutrients while the red nodes visualize upregulated toxic metals. (B) Cell viability of MCF10A cells treated with fruit (top) and leaf (bottom) extracts for 24 h and 48 h. Extracts from olives grown at different distances: T (closest), C (middle), D (farthest).