Systematic engineering for production of anti-aging sunscreen compound in *Pseudomonas putida*

Background/Objective

- Most commercial sunscreen contains compounds that raise concerns due to their negative effects on human health and the environment.
- Shinorine is a mycosporine-like amino acid and an active ingredient in commercial sunscreen product.
- We aim to establish an efficient microbial platform for production of shinorine using *P. putida*.

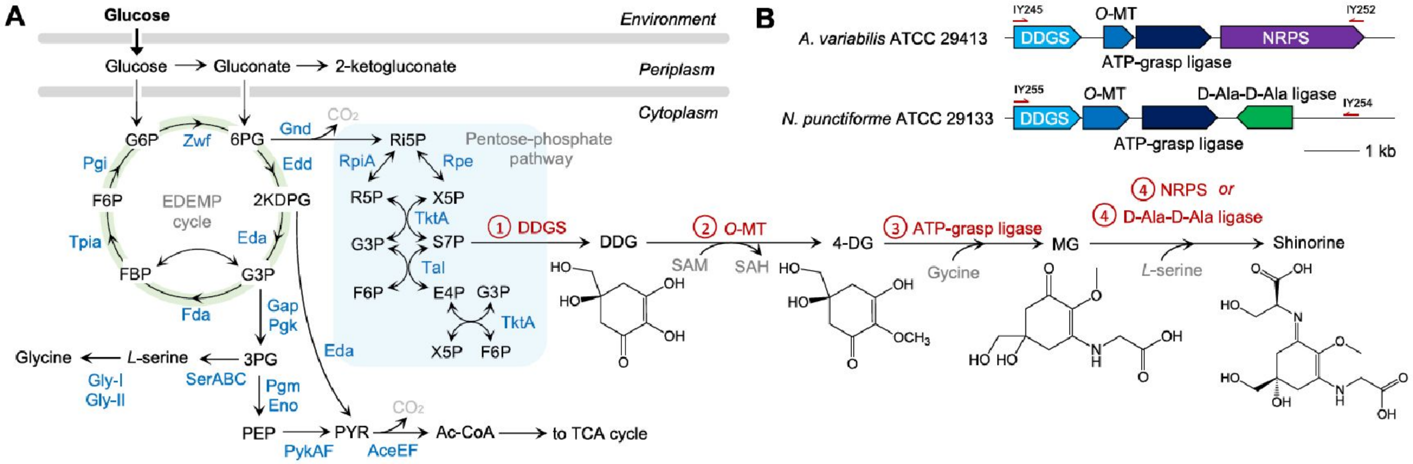


Figure 1. Production experiment of shinorine in engineered *P. putida* KT2440. (A) Schematic diagram of *P. putida* KT2440 central carbon metabolism and shinorine biosynthetic pathway. (B) Shinorine biosynthetic gene cluster (BGC) from *Anabaena variabilis* and *Nostoc punctiforme*.

Approach

- We engineered metabolically versatile host *P. putida* for shinorine production.
- We employed computational modeling, synthetic biology tools such as CRISPRi and RBS engineering to maximize shinorine production in *P. putida*.

Results

- We analyzed central carbon flux distribution data from GEMs and selected *P. putida* as an appropriate host for shinorine production.
- We enhanced shinorine productivity through CRISPRi-mediated downregulation, proteomics, RBS and promoter engineering and amino acid supplementation.

Significance/Impacts

- Our study combines multiple approaches to improve shinorine production, demonstrating the potential of *Pseudomonas putida* KT2440 as a platform for microbial cell factories.

Yunus, I., et. al. Metabolic Engineering. doi: 10.1016/j.ymben.2024.06.001

Engineered plants provide a photosynthetic platform for the production of diverse human milk oligosaccharides

Background/Objective

- Human milk oligosaccharides (HMOs) are a diverse class of carbohydrates that aid in the health and development of infants.
- The vast health benefits of HMOs have made them a commercial target for microbial production; however, producing the ~200 structurally diverse HMOs at scale has proven difficult.

Approach

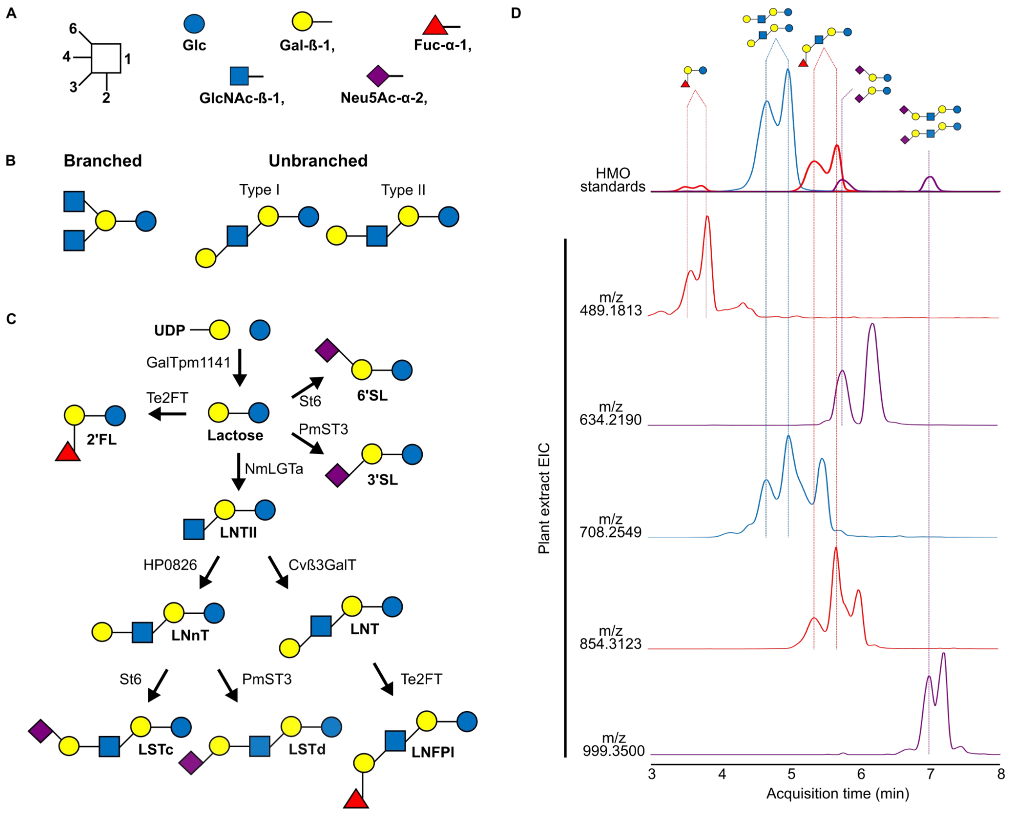
Here, we produce a vast diversity of HMOs by leveraging the robust carbohydrate anabolism of plants

Results

We demonstrated that plants are highly effective at making a range of high value and complex HMOs.

Significance/Impacts

Technoeconomic analyses demonstrate that producing HMOs in plants provides a path to the large-scale production of specific HMOs at lower prices than microbial production platforms.



Barnum C. R., et. al. Nature food. doi: 10.1038/s43016-024-00996-x

Systematic identification of transcriptional activation domains from non-transcription factor proteins in plants and yeast

Background/Objective

Transcription factors can promote gene expression through activation domains. Whole-genome screens have systematically mapped activation domains in transcription factors, but not in non-transcription factor proteins (e.g., chromatin regulators, coactivators).

Approach

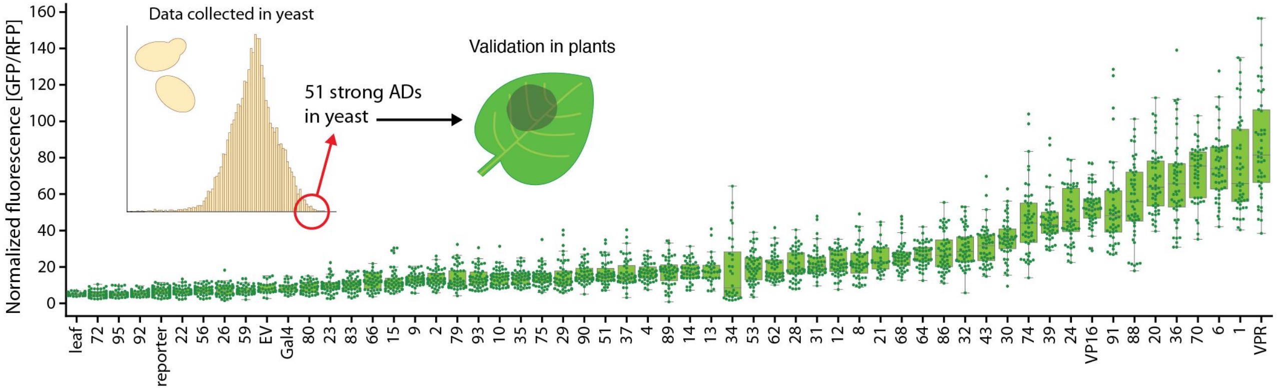
We used a machine learning algorithm to analyze the proteomes of Arabidopsis thaliana and Saccharomyces cerevisiae. We screened 18,000 predicted activation domains from >800 non-transcription factor genes in both species, confirming that 89% of candidate proteins contain active fragments.

Results

Our work enables the annotation of hundreds of nuclear proteins as putative coactivators, many of which have never been ascribed any function in plants. We validated short, 'universal' activation domains with comparable performance to state-of-the-art activation domains used for genome engineering.

Significance/Impacts

Our approach enables the genome-wide discovery and annotation of activation domains that can function across diverse eukaryotes.



Hummel N. F. C., et. al. Cell systems. doi: 10.1016/j.cels.2024.05.007

JBEI
Joint BioEnergy Institute

Lignin engineering in poplar via heterologous expression of dehydroshikimate dehydratase induces distinct transcriptional and metabolic changes in the shikimate and phenylpropanoid pathways

Background/Objective

- In-planta expression of a 3-dehydroshikimate dehydratase (QsuB) in poplar reduces lignin content
- Understanding how plants respond at the transcriptional and metabolic levels is needed to facilitate further improvement and field deployment.

Approach

we generated fundamental knowledge on lignin-modified QsuB poplar using RNA-seq and metabolomics

Results

Changes in gene expression and metabolite reveal tissue-specific responses in the xylem, phloem, or periderm.

These result in the accumulation of hydroxybenzoates derived from protocatechuate and salicylate.

Significance/Impacts

In addition to its reduced recalcitrance to enzymatic deconstruction, QsuB-poplar biomass is a source of valuable and simple aromatics

Akyuz Turumtay E., et. al. J. Exp. Bot. doi: 10.1093/jxb/erae251

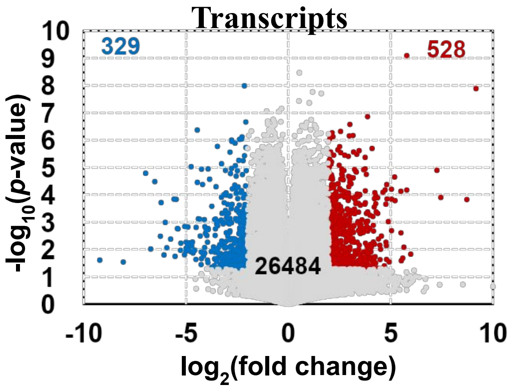


Figure 1. Volcano plot of transcripts identified in the xylem from wildtype and QsuB poplar. The number of downregulated (in blue) and upregulated (in red) transcripts in QsuB poplar is indicated (log2-fold change +2/-2 and p-value < 0.05). Gray dots represent transcripts not differentially expressed

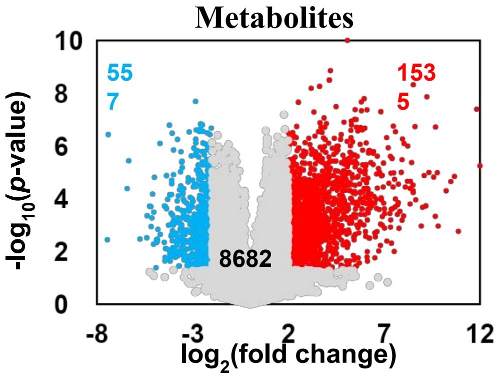


Figure 2. Volcano plot of metabolites identified in the xylem from wildtype and QsuB poplar. The number of less abundant (in blue) and more abundant (in red) metabolites in QsuB poplar is indicated (log2-fold change +2/-2 and p-value < 0.05). Gray dots represent unchanged metabolites.

JBEI
Joint BioEnergy Institute

Toward a Circular Bioeconomy: Designing Microbes and Polymers for Biodegradation

Background/Objective

This review discusses the biodegradation of common synthetic polymers, identifies novel pathways to metabolize recalcitrant materials and engineering strategies to enhance these pathways.

Approach

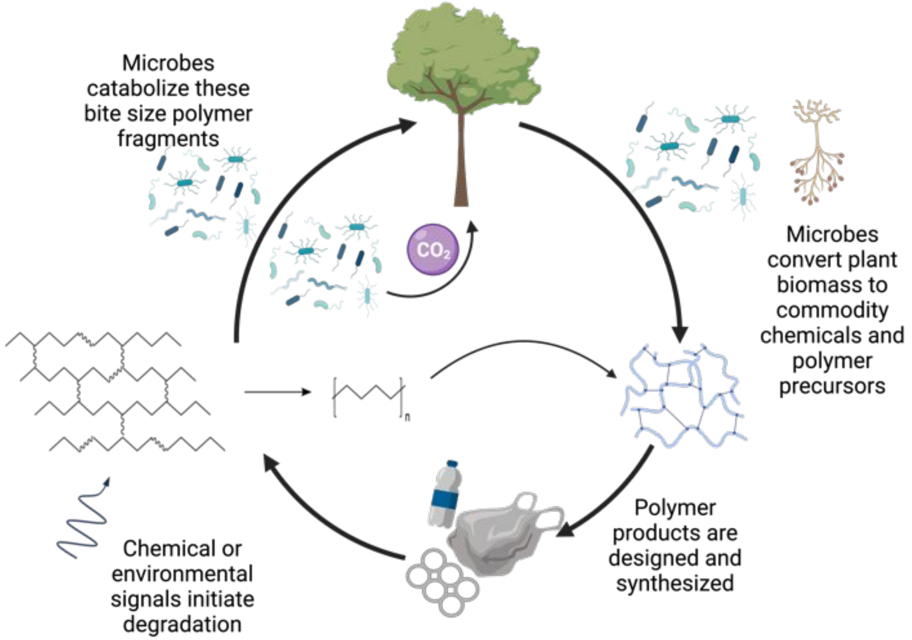
We delved into recent literature to identify some of the most important discoveries that can enhance bioprocessing of common recalcitrant polymers

Results

We reviewed novel microbes with strong potential to improve the biodegradation of synthetic polymers, and drew a connection between lignin biodegradation and the biodegradation of synthetic polymers

Significance/Impacts

Materials scientists, bioengineers, and chemists should consult each other when designing future generations of polymers to design materials that can biodegrade at the end of its life.



Microbes catabolize these bite size polymer fragments

Microbes convert plant biomass to commodity chemicals and polymer precursors

Polymer products are designed and synthesized

Chemical or environmental signals initiate degradation

A vision to achieve a circular carbon life cycle. Microbes convert plant biomass to value-added biopolymers, which can be converted back to polymer precursors or broken down and metabolized by more microbes at the end of their lives, releasing metabolites and carbon dioxide to contribute to the growth of new plants.

Mubayi V., et. al. ACS synthetic biology. doi: 10.1021/acssynbio.4c00077

ENABLED PUBLICATIONS

JBEI
Joint BioEnergy Institute

Metagenomics analysis yields assembled genomes from prokaryotic anaerobes with polymer-degrading potential

Background/Objective

- Anaerobic microbial consortia from degradative environments have not been sufficiently characterized
- Microbes that degrade recalcitrant polymers, like lignin, may be able to degrade plastics

Approach

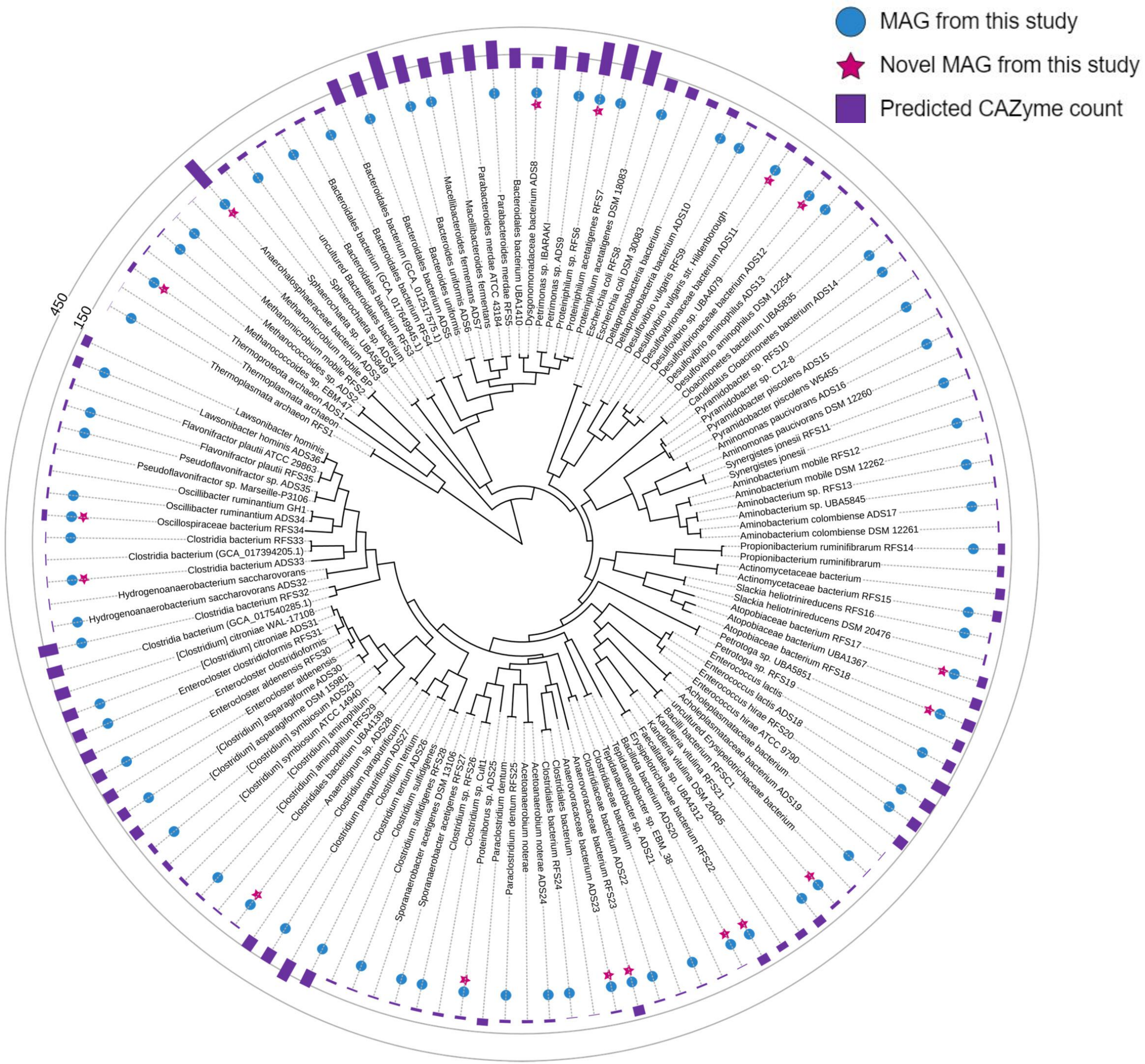
We cultivated microbial consortia from goat feces and anaerobic digester sludge in the presence of plastics and sequenced the DNA.

Results

We assembled several MAGs from previously uncharacterized species and found microbes with high degradative potential.

Significance/Impacts

These MAGs can be further mined and provide insight into these degradative environments.



Blair E. M., et. al. Biotechnology progress. doi: 10.1002/btpr.3484

The elite haplotype OsGATA8-H coordinates nitrogen uptake and productive tiller formation in rice

Background/Objective

Excessive nitrogen promotes the formation of non-productive tillers in rice, which decreases nitrogen use efficiency (NUE). Developing high-NUE cultivars with balanced nitrogen uptake and productive tillers remains a long-standing challenge.

Approach

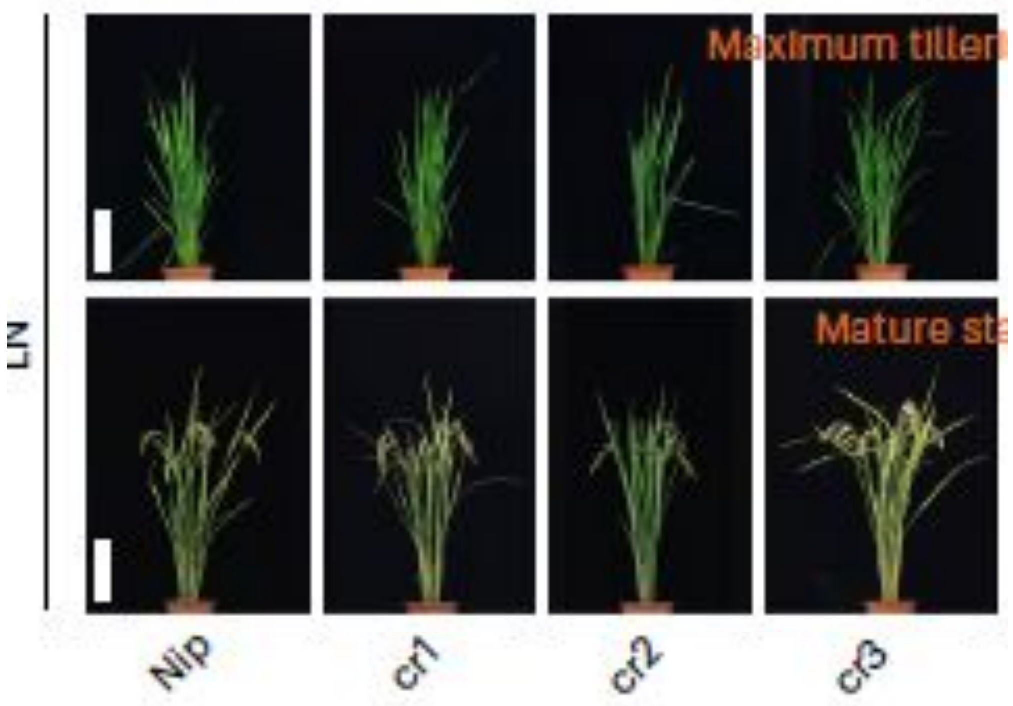
We identified the transcription factor OsGATA8 as a key coordinator of nitrogen uptake and tiller formation in rice.

Results

We discovered OsGATA8-H as a high-NUE haplotype with enhanced nitrogen uptake and a higher proportion of productive tillers.

Significance/Impacts

- The geographical distribution of OsGATA8-H and its frequency change in historical accessions suggests adaption to fertile soil.
- Creation of OsGATA8 promoter alleles that functionally resemble OsGATA8-H via genome editing offers a possible strategy to generate sorghum with optimized NUE.



Compared with the wild-type (Nip) plants, both PTNR and the proportion of productive tillers were higher in the OsGATA8 knockout plants These results indicate that OsGATA8 is a negative regulator of PTNR and the proportion of productive tillers under low nitrogen conditions.

Wu W., et. al. Nature genetics. doi: 10.1038/s41588-024-01795-7

Evolution and engineering of pathways for aromatic O-demethylation in Pseudomonas putida KT2440

Background

- Depolymerization of lignin, followed by bioconversion in *Pseuduomonas putida* KT2440, is a promising approach to convert lignin to valuable products.
- Methoxylated monomers like vanillate must undergo *O*-demethylation to enter catabolic pathways. Several enzyme families catalyze aromatic *O*-demethylation, but they are rarely compared *in vivo* to determine an optimal biocatalytic strategy.

Approach

- Vanillate *O*-demethylation pathways were compared in engineered strains of *P. putida*, focusing on the LigM and VanAB systems (**Figure 1**).
- Adaptive laboratory evolution optimized each pathway, and mutations were identified by next-generation sequencing.

Results

All evolved strains displayed improved growth phenotypes (**Figure 2**), but those harboring the optimized VanAB pathway demonstrated faster vanillate utilization.

Significance

- Illuminates the details of vanillate catabolism in the context of two distinct pathways.
- Yields a platform strain for efficient conversion of vanillate to value-added products.

Bleem A. C., et. al. Metabolic engineering. doi: 10.1016/j.ymben.2024.06.009

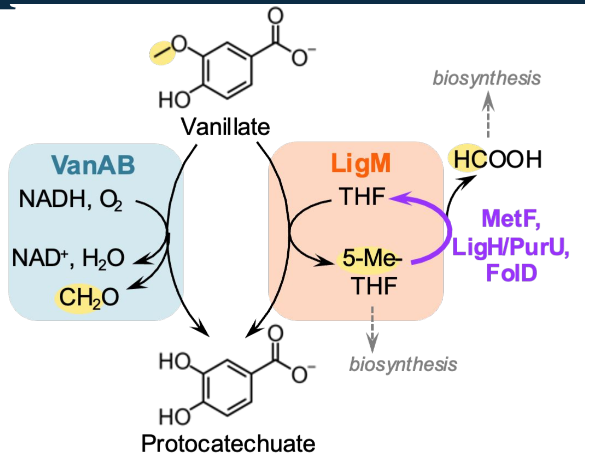


Figure 1. Two unique mechanisms for vanillate *O*-demethylation were investigated in *P. putida*

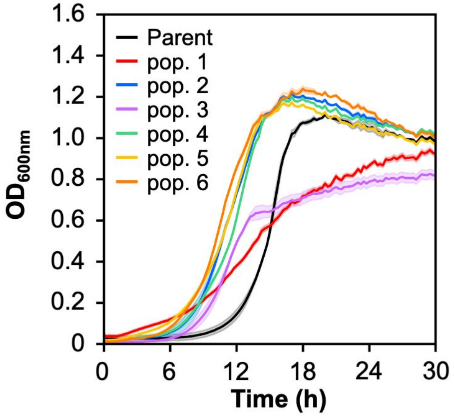


Figure 2. Evolved populations (colors) exhibited improved growth with vanillate, compared to the parent strains (black).

Recommendations for life-cycle assessment of recyclable plastics in a circular economy

Background/Objective

- Plastics are ubiquitous and, next to fuels, the largest-volume product from petroleum
- Bio-based alternatives and novel recycling strategies need to be compared and prioritized

Approach

Critical reviewed life-cycle assessment methods applied to conventional and bio-based plastic production as well as recycling

Results

GHG emissions are not the most important impact of replacing conventional plastics. Other impacts, such as microplastics emissions, ecological impacts, and system-wide burdens on waste management are more important

Significance/Impacts

A more comprehensive framework can enhance the value proposition for novel polymers and recycling strategies.

Nordahl S. L., et. al. Chemical science. doi: 10.1039/d4sc01340a

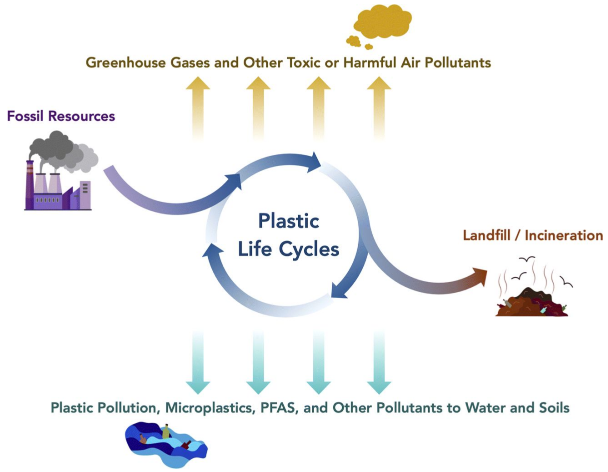


Figure 1: Environmental impacts from plastic life cycles. PFAS refer to per-and polyfluoroalkyl substances.

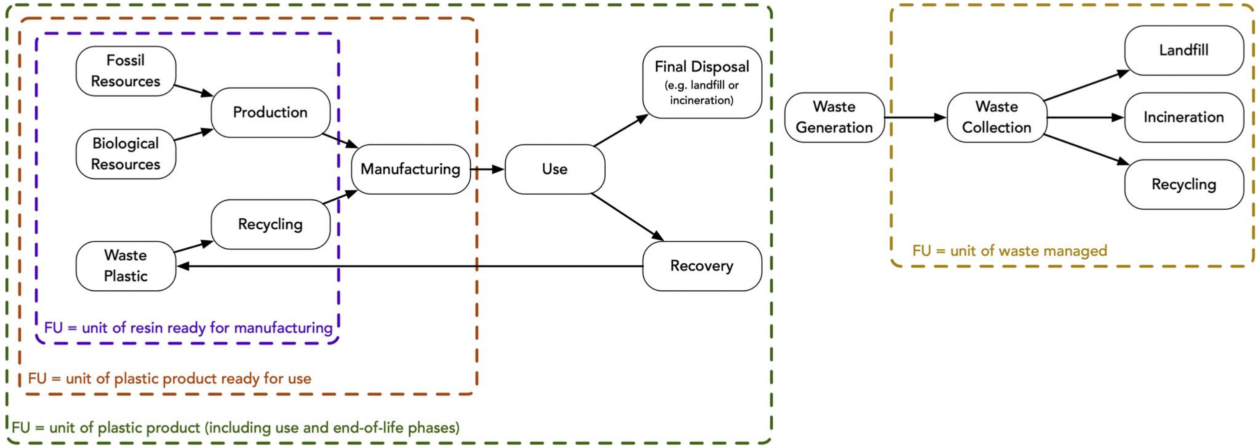


Figure 2: Example system boundaries for various functional units (FU: functional unit).