

Microbial valorization of lignin to malic acid by *Aspergillus niger*

Background/Objective

- Lignin is the largest source of aromatic carbon in nature, and it is an abundant feedstock for producing bio-based fuels and chemicals.
- Bioprocesses to convert lignin into valuable products are needed.

Approach

- *Aspergillus niger* was engineered to enable production of malic acid from lignin-derived aromatics, and culture conditions were optimized to produce malic acid from depolymerized lignin.

Results

- The engineered *A. niger* produced 3.5 - 3.9 g/L of malic acid from 10 g/L of lignin-derived aromatic monomers in a minimal medium.
- Depolymerized poplar and sorghum lignin was converted to malic acid by the engineered *A. niger* in the optimized culture conditions.

Significance/Impacts

- Fungal bioconversion of lignin to malic acid demonstrates its potential for enhancing process integration and economic feasibility for biorefineries.

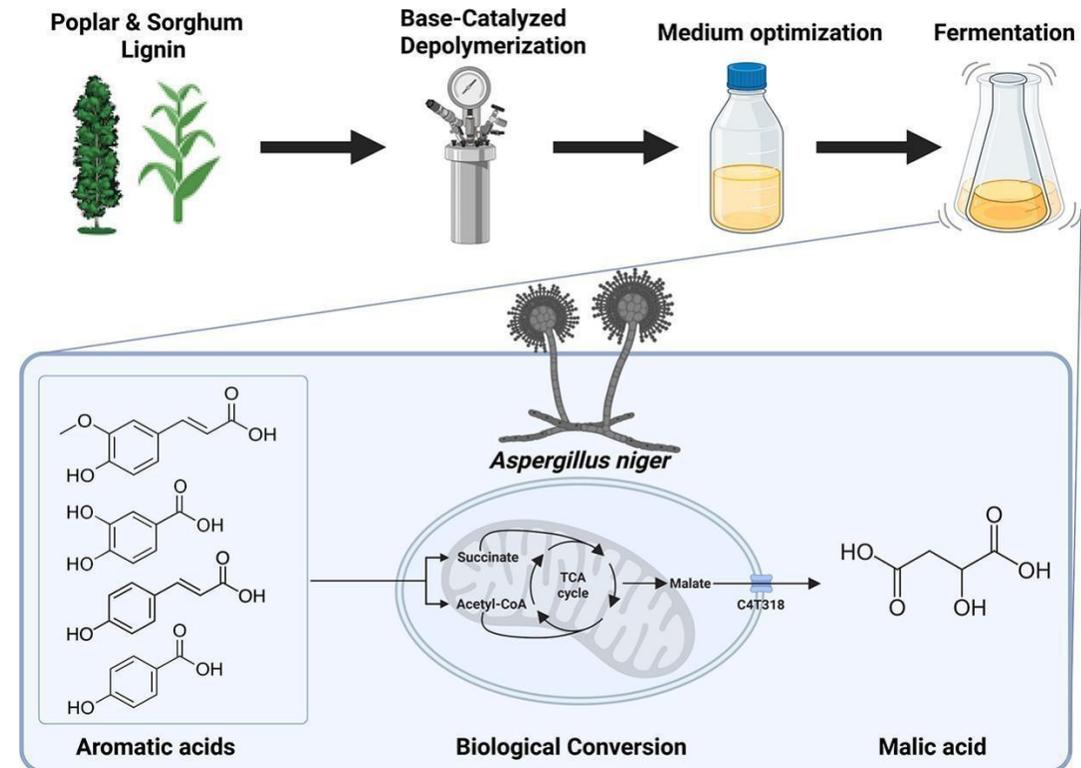


Figure caption: Fungal bioconversion of depolymerized lignin into malic acid. The aromatic acids from base-catalyzed depolymerization of poplar and sorghum lignin were converted to malic acid by engineered *Aspergillus niger* in optimized culture conditions.

Rapid Evaluation of Amine-Functionalized Solvents for Biomass Deconstruction Using High-Throughput Screening and One-Pot Enzymatic Saccharification

Background/Objective

- Efficient pretreatment of lignocellulosic biomass is a bottleneck for economical biofuel and biochemical production. Traditional optimization is slow and resource heavy. This work aims to create a rapid, miniaturized screening method to evaluate amine-functionalized solvents for biomass deconstruction across different feedstocks.

Approach

- Built a custom high-throughput screening (HTX) platform that integrates solvent pretreatment, vacuum drying, and one-pot enzymatic saccharification.
- Screened five amine-functionalized solvents on sorghum, poplar, and switchgrass at varied pretreatment temperatures to compare sugar release and solvent removal efficiency.

Results

- Isopropanolamine and N-methylbutylamine gave the best results, yielding 70-80% glucose and 58-67% xylose. Vacuum drying removed > 99 % solvent, eliminating water wash steps. Hydrolysates supported growth of engineered *Rhodospiridium toruloides*, showing biocompatibility.

Significance/Impacts

- This HTX platform enables rapid, scalable, feedstock-agnostic evaluation of pretreatment solvents, speeding solvent-feedstock pairing and process optimization for cost-competitive biorefineries. It bridges pretreatment and biological conversion in a single workflow.

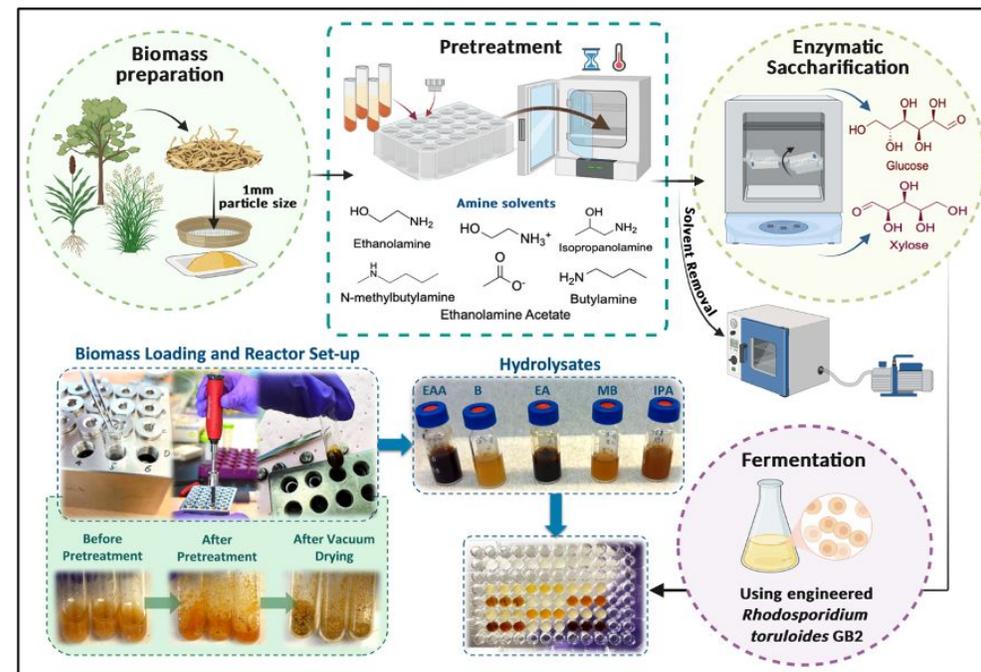


Figure caption: Schematic representation of the experimental workflow used for high-throughput solvent screening comprising biomass preparation, pretreatment, saccharification and fermentation steps.

Engineered Production of Hydroxycinnamoyl Tyramine Conjugates Limits the Growth of the Pathogen *Pseudomonas syringae* in *Arabidopsis*

Background/Objective

- Hydroxycinnamoyl tyramine conjugates are phytoalexins synthesized during wounding and pathogen attack in many species.
- The objective was to investigate the overexpressing TyDC/THT effects on cell wall structure and plant immunity.

Approach

- Tyramine conjugates such as p-coumaroyltyramine (CT) and feruloyltyramine (FT) overexpressed in *Arabidopsis*.
- Metabolite and transcriptome changes and pathogen response were measured.

Results

- Overproduction of CT and FT in *Arabidopsis* leads to altered expression of genes regulating circadian rhythms. The reduction of *P. syringae* growth in rosettes supports a crosstalk between the circadian clock and immunity.

Significance/Impacts

- Our findings suggest a novel role for hydroxycinnamoyl tyramine conjugates in pathogen resistance by modulating the biological circadian clock.

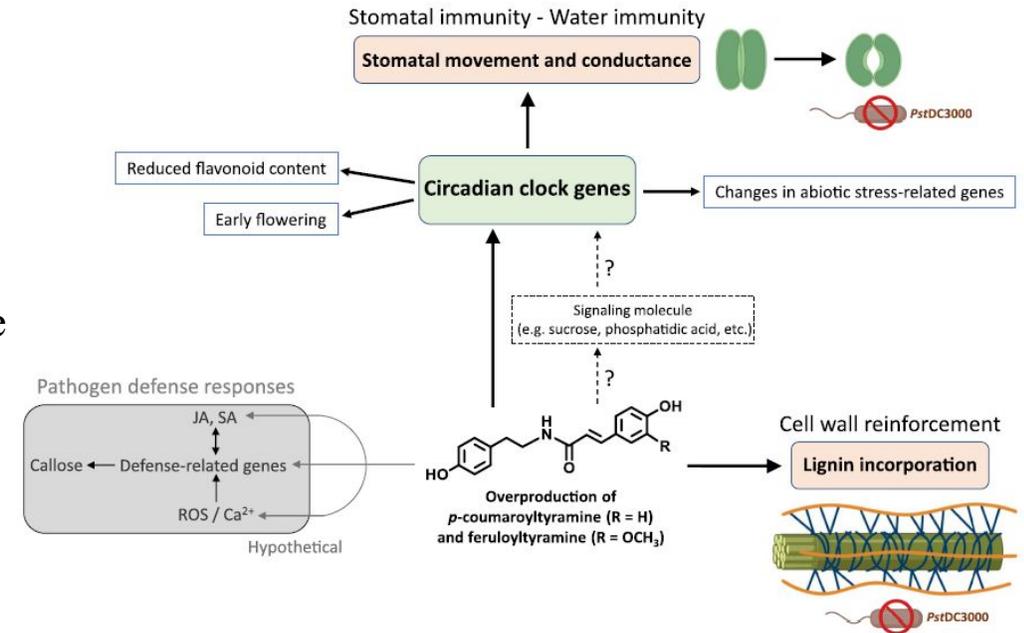


Figure caption: Proposed modes of action for hydroxycinnamoyl tyramine conjugates (HTCs) in transgenic *Arabidopsis* involve feruloyltyramine (FT) partially incorporating into lignin and both FT and p-coumaroyltyramine (CT) modifying circadian clock genes/altering stomatal conductance. These effects may inhibit PstDC3000 by potentially reinforcing cell walls and enhancing stomatal/water immunity. The mechanism for gene expression influence is unknown. FT and CT did not activate innate immunity (no elevated JA, SA, ROS, callose, or defense gene induction).

Reduced methane emissions in transgenic rice genotypes are associated with altered rhizosphere microbial hydrogen cycling

Background/Objective

- PSI peptides are endogenous signaling factors in plants involved in controlling root growth. OsPSY1 overexpressors have longer roots.
- The objective was to investigate the effect of overexpressing *OsPSY1* on rhizosphere interactions.

Approach

- OsPSY1 was overexpressed in rice.
- Growth, microbiome metatranscriptomics and methane emissions were measured.

Results

- Plants overexpressing OsPSY1 emitted 50% less methane
- The OsPSY1 overexpressors stimulated microorganismal activity predicted to result in reduced hydrogen production in the rhizosphere and less methane generation.

Significance/Impacts

- The altered rhizosphere microbial activity in PSY1 overexpressing plants will affect release of methane and reduce the environmental impact of rice cultivation.
- Altered exudate profiles in PSY1 overexpressors are likely to impact rhizosphere interactions in other species, including sorghum.

Shi, L. D., et.al. Nature communications. 10.1038/s41467-026-68640-9 (JBEI #1297)

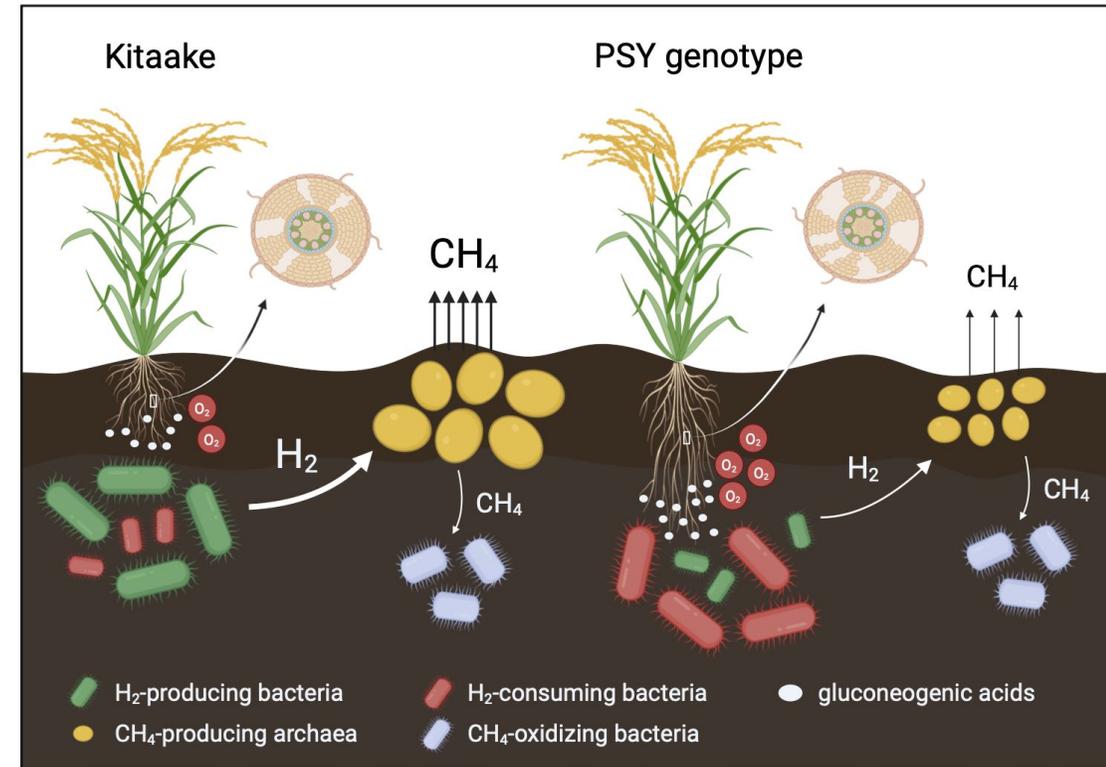


Figure caption: Proposed mechanism for the role of PSY rice genotypes in mitigating methane emissions. PSY rice genotypes have longer roots with more aerenchyma compared with control plants (Kitaake). O₂ is transported from the atmosphere to the rhizosphere through aerenchyma. PSY rice roots secrete more gluconeogenic acids (white circles), mostly amino acids, that can facilitate the activities of H₂-consuming bacteria (indicated by cell sizes), thus reducing the total H₂ pool for H₂-dependent methanogenesis. The produced methane was partially oxidized in situ by methanotrophic bacteria and the other was emitted into the atmosphere.

Enabled Publications

The Potential and Cost of Carbon Dioxide Removal Using Direct Air Capture with Land-Based Wind and Utility-Scale Photovoltaics

Background/Objective

- The land footprint of new energy and carbon management technologies is a key constraint in their build-out. JBEI has developed land cover models that are versatile in their application.

Approach

- We contributed detailed land cover data for use in developing scenarios for the build-out of direct air capture co-located with wind and solar generation.

Results

- There is technical potential for low-temperature, adsorbent-based DACS to remove approximately 9 gigatonnes of CO₂ annually
- Agricultural land offers substantial capacity for co-location of solar/wind.

Significance/Impacts

- Multi-use scenarios for land cover, particularly in regions with marginal agricultural land are key to maximizing value and finding synergies between bioenergy and other forms of energy and carbon management.

Hunter-Sellars, E., et.al. Environmental science & technology. 10.1021/acs.est.5c14628(JBEI #131)

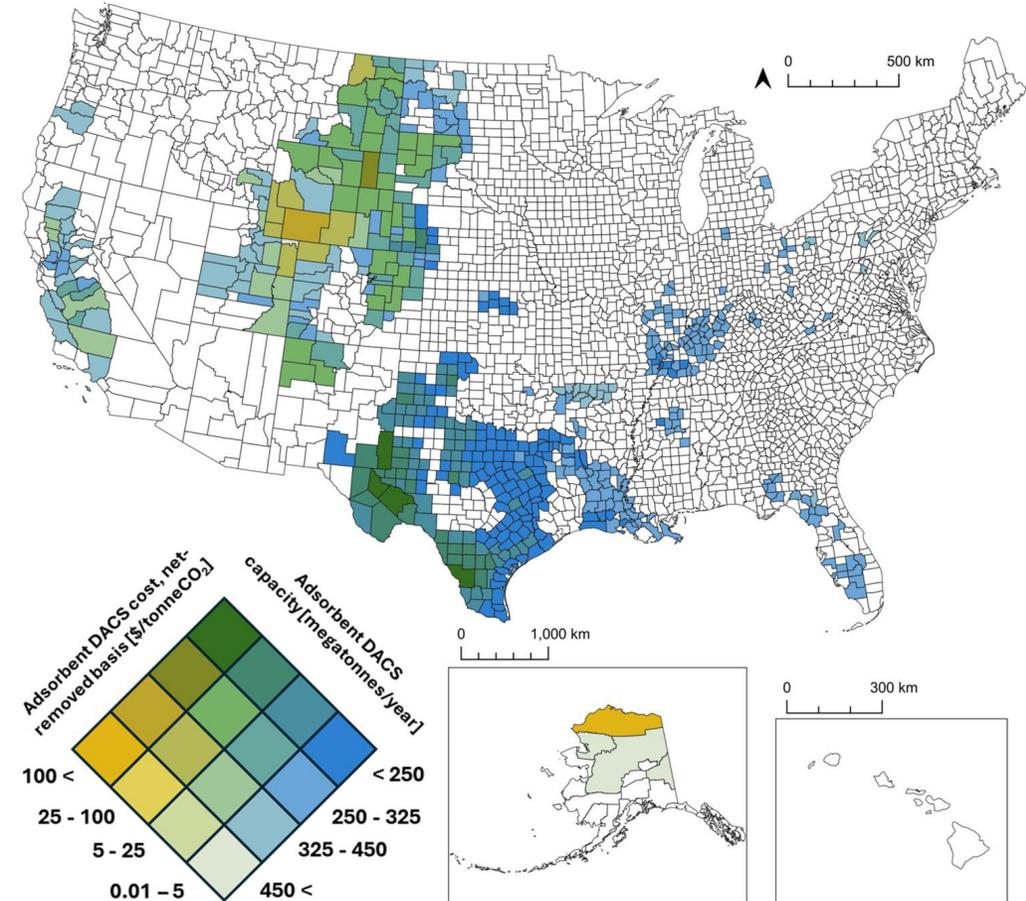


Figure caption: Potential and projected costs for direct air capture co-located with wind and solar photovoltaic generation, taking advantage of potential for installation on agricultural lands