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Ectopic expression of pectate lyase PtxtPL1-27 in aspen affects leaf cuticle development

Background/Objective

- The cuticle is a barrier covering outer cell surfaces of plants
- It has been unclear how hydrophobic cuticle components travel through the cell wall to form cuticle on the surface

Approach

- A pectate lyase was expressed in *Populus* under 35S promoter
- Transgenic plants were phenotyped using chemical and microscopy analyses

Results

- Overexpression of pectate lyase led to reduced pectin content in cell walls
- Cuticle thickness was drastically reduced in the transgenic plants
- Cutin and lipids accumulated in the walls of transgenic plants

Significance/Impacts

- The cuticle is a critical barrier protecting plants against abiotic and biotic stress
- Understanding how cuticle deposition is regulated could enable development of more resilient bioenergy crops
- Understanding the impact of biomass composition on cuticle is important to avoid undesirable effects of biomass modification e.g. for increased saccharification

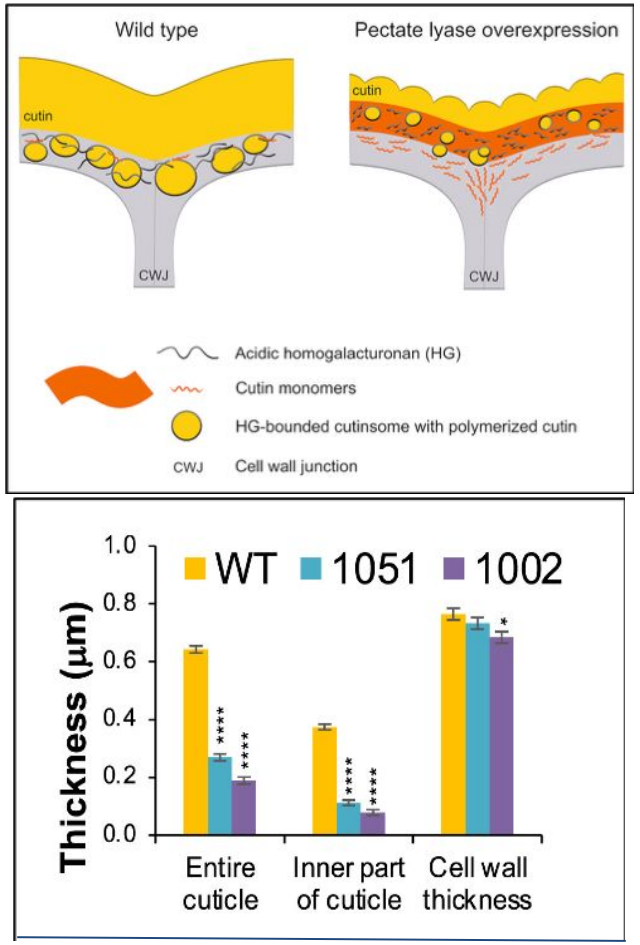

Figure caption: **Top**: Schematic illustration of the effect of pectate lyase overexpression in cell wall and cuticle. Cutinosomes are vesicles that transport cuticle components and deposit them to form the cuticle. **Bottom:** Thickness of cuticle and epidermal cell wall in wild type and two transgenic lines.

Biswal A.K., et.al. iScience. DOI: 10.1016/j.isci.2025.113963 (JBEI #1276)

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The ‘photosynthetic C1 pathway’ links carbon assimilation and growth in California poplar

Background/Objective

- While photosynthesis forms the basis of the global carbon cycle, the detailed biochemical mechanisms remain under investigation
- Although ecosystems worldwide show increased photosynthesis and growth under elevated CO_2, the specific mechanisms remain unclear, with essential nutrients like nitrogen often viewed as a limiting factor in the 'CO_2 fertilization' effect

Results

- We discovered a highly active photosynthetic C_1 pathway in poplar trees, which assimilates the raw ingredients of life (CO_2, NH_3 and SO_4^{-2}) in the light
- This process integrates CO_2 assimilation directly into C_1 metabolism, supporting biomass growth with a strongly ^{13}C-depleted signature
- We highlight that C_1 photosynthesis could be widespread across the plant kingdom
- •Plant methanol emissions were also found to act as a non-invasive chemical sensor of diurnal growth processes

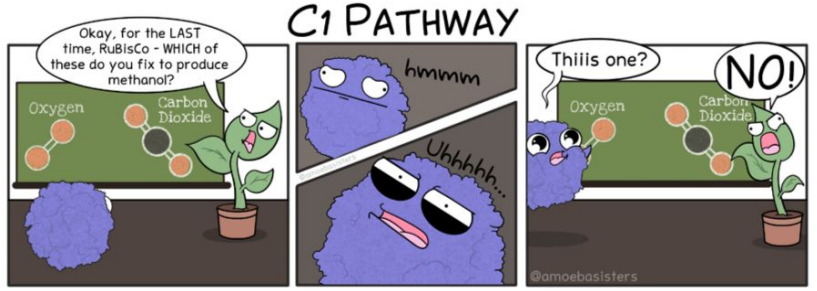

Figure 1: The ‘**photosynthetic C_1 pathway’**, rather than photorespiration, leads to the generation of light-dependent serine and methionine synthesis used as a source of methyl transfer reaction during growth and development.

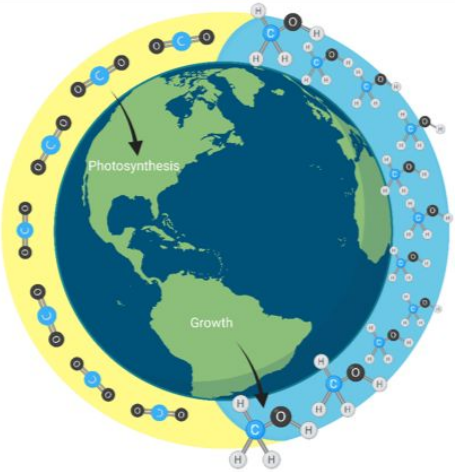

Figure 2: Graphical simplification of the role of the photosynthetic C_1 pathway in supporting CO_2 fixation, plant growth, and methanol (CH_3OH) emissions in global biosphere-atmosphere interactions. [C_1 photosynthesis animation]

Significance/Impacts

- C_1 photosynthesis pathway may be a fundamental mechanism in the evolution of life on Earth, representing a missing link between changing atmospheric composition due to human activities and ‘CO_2 fertilization’ of the biosphere
- The discovery also sheds light on plant-produced methane, suggesting it is not just transported from the soil but also synthesized directly in photosynthetic tissues
- The findings are also expected to contribute to genetic engineering efforts aimed at enhancing photosynthesis rates and plant productivity

Jardine, K.J., et.al. Commun Biol. DOI: 10.1038/s42003-024-07142-0 (JBEI #1277)

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Mechanistic Insights into the Demethylation of Lignin-Derived Structures Using Protic Ionic Liquids: A Density Functional Theory Study

Background/Objective

- Lignin's methoxy groups limit reactivity; efficient demethylation is needed to unlock high-value lignin upgrading. Traditional demethylation uses harsh acids; greener catalytic systems are needed
- Understand how PIL–water environments control energetics and selectivity of demethylation

Approach

- Combined COSMO-RS screening with DFT (implicit + explicit solvation) to evaluate PIL–water interactions
- Computed reaction pathways, TS structures, and activation energies for guaiacol and syringol; performed HOMO-LUMO, charge-transfer, and MoEP analyses

Results

- Monoethanolamine acetate showed strongest stabilization, reducing demet-hylation barriers by >30 kcal/mol compared to water alone
- Guaiacol reacts more readily than syringol due to lower steric hindrance and more favorable charge redistribution
- Increasing acetic acid concentration (1:1 → 1:4) further lowered activation barriers via enhanced proton-relay networks

Significance/Impacts

- Provides first atomic-level basis for designing PIL compositions that tune lignin demethylation selectivity
- Offers solvent-design principles for scalable biomass conversion technologies

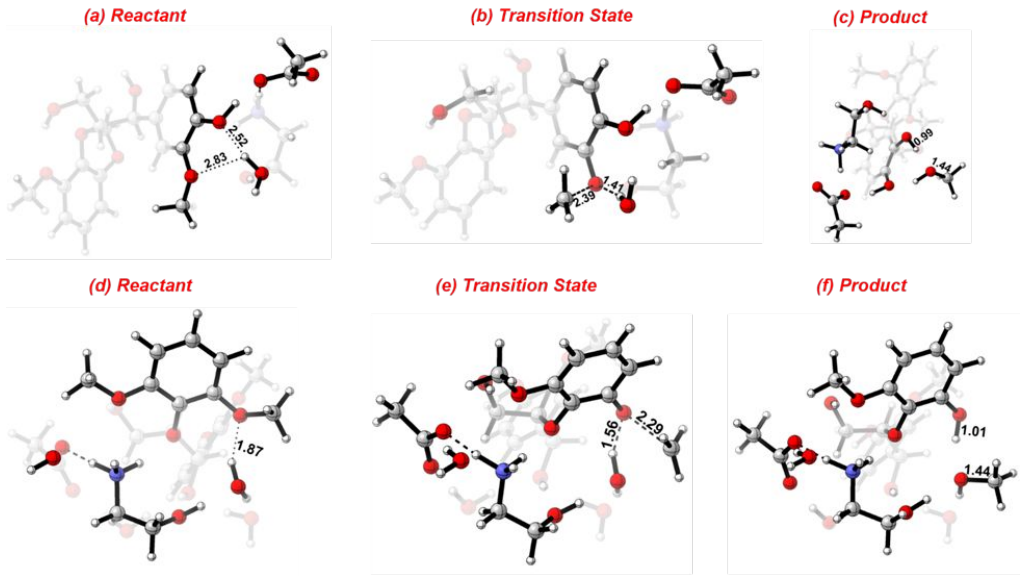

Figure caption: Optimized structures along the demethylation pathways in the presence of the protic ionic liquid and water-bridged complex. (a)-(c) Reactant, transition state, and product for the G unit. (d)-(f) Reactant, transition state, and product for the S unit. Key bond distances are labeled in Å.

Mishra D.K., et.al. Chemphyschem. DOI: 10.1002/cphc.202500374 (JBEI #1278)

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Engineering Polyketide Stereocenters with Ketoreductase Domain Exchanges

Objective: Control stereochemistry of unnatural products

- Polyketide synthases (PKSs) can make a large range of natural and unnatural products with rich stereocenters

Approach: KR domain exchanges

- Used bioinformatics / ClusterCAD to find KR domains with desired stereochemistry and make unnatural products

Results: Our strategy allowed us to regulate stereocenters

- We made all possible stereochemical configurations with 3 different PKSs and in 2 different host organisms

Significance: We can precisely engineer PKS products

- Biofuels and bioproducts require specific stereochemistry to work as intended

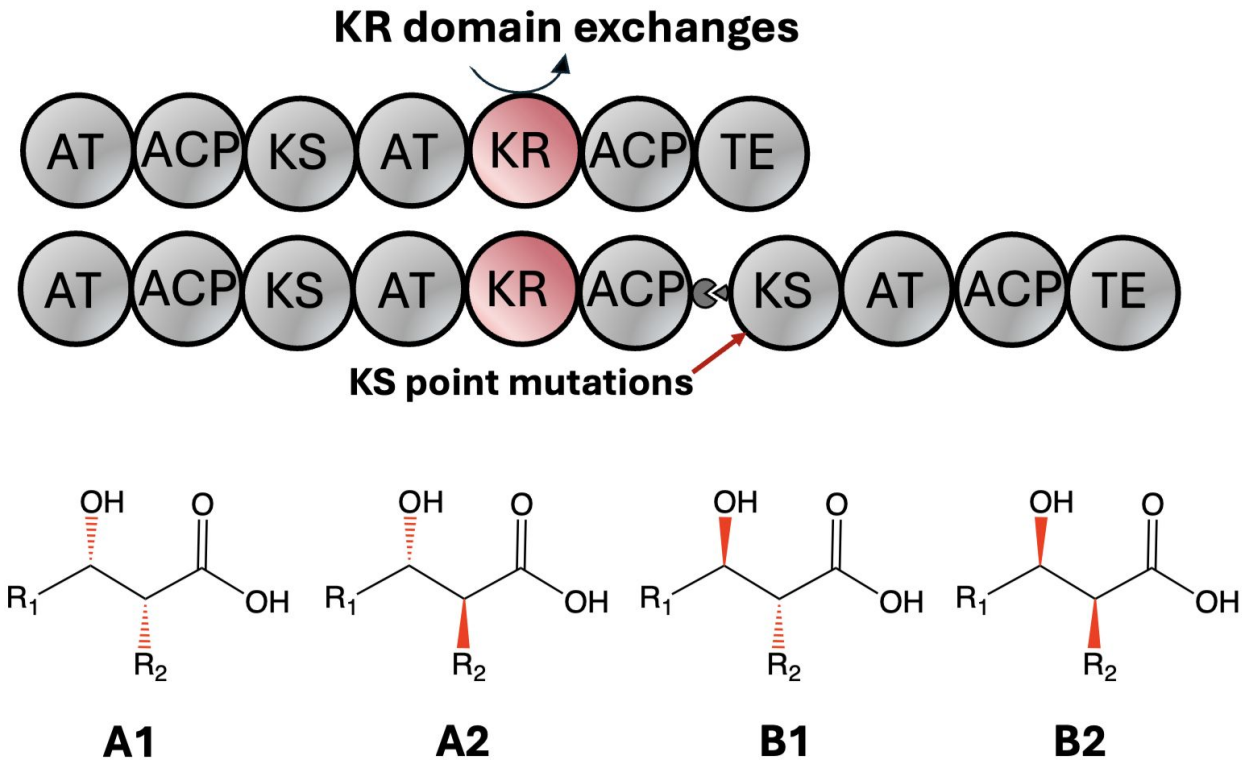

Figure caption: In this study, we systematically tested and evaluated strategies for ketoreductase (KR) domain exchanges, the domain responsible for setting stereocenters of polyketide products. After first optimizing the method for KR exchanges, we then performed 44 KR domain exchanges on three different PKSs to obtain high production of all four stereoisomers in vivo.

Keiser L.S., et.al. J Am Chem Soc. DOI: 10.1021/jacs.5c06736 (JBEI #1279)

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Engineered plants for the production of the antioxidants arbutin and gallate

Background/Objective

- Biomass value can be increased via the accumulation of useful bioproducts
- Arbutin and gallate are two valuable antioxidants as well as potential polymer precursors
- The objective was to overproduce arbutin and gallate in plant biomass

Approach

- Microbial genes were expressed in plants to reroute intermediates of the shikimate pathway towards arbutin and gallate production (Figure 1)

Results

- Gallate and arbutin titers of 0.58 dwt% and 0.50 dwt% were achieved in the bioenergy crop sorghum (Figure 2)
- Gallate and arbutin are readily extracted from biomass using aqueous methanol solvent and without altering fibers (i.e. cell walls)

Significance/Impacts

- This study presents alternative synthesis routes using plant hosts for the production of gallate and arbutin
- Purifying arbutin and gallate from engineered biomass could improve the economics of advanced biofuels and bioproducts

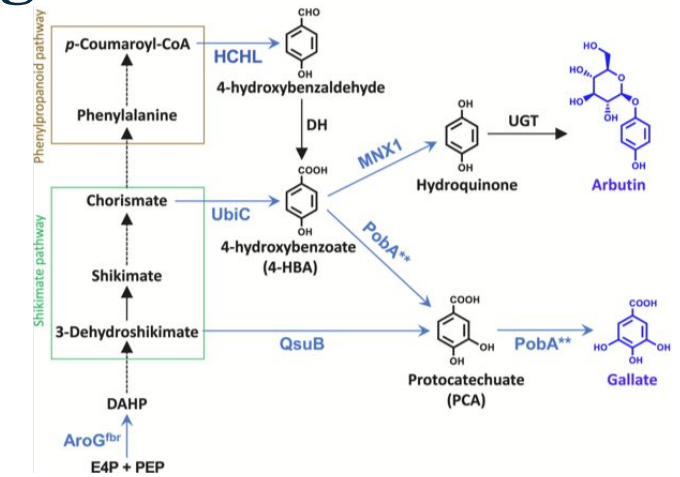

Figure 1: Metabolic routes for gallate and arbutin synthesis. Heterologous enzymes (in blue) are: AroG^{fbr}, feedback-resistant 3-deoxy-D-arabinoheptulosonate 7-phosphate synthase; HCHL, hydroxycinnamoyl-CoA hydratase-lyase; MNX1, 4-hydroxybenzoate 1-hydroxylase; PobA**, 4-hydroxybenzoate 3,5-hydroxylase; QsuB, 3-dehydroshikimate dehydratase

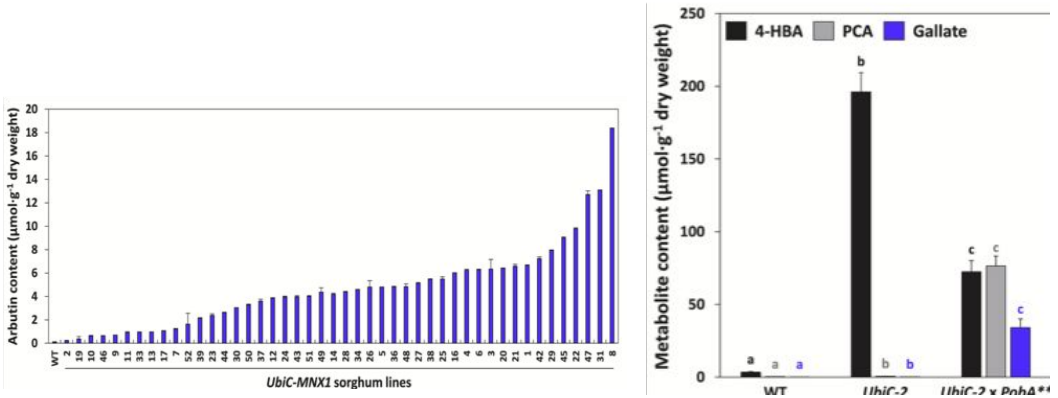

Figure 2: Titers of arbutin (left) and gallate (right) produced in leaf and stem biomass from mature engineered sorghum plants.

Kazaz S., et.al. Metab Eng. DOI: 10.1016/j.ymben.2025.11.009 (JBEI #1280)

Predictive CRISPR-mediated gene downregulation for enhanced production of sustainable aviation fuel precursor in Pseudomonas putida

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Background/Objective

- CRISPR interference (CRISPRi) is a valuable tool for redirecting metabolic flux to enhance bioproduction
- Its application is often constrained by two challenges: (i) rationally identifying effective gene targets for downregulation and (ii) efficiently constructing multiplexed CRISPRi systems

Approach

- A computational prioritization tool (FluxRETAP (Flux-Reaction Target Prioritization)) was used for knock-down targets selection
- And VAMMPIRE (Versatile Assembly Method for MultiPlexing CRISPRi-mediated downREgulation) was used to build multiplexed gRNA systems to improve isoprenol production in *P. putida*

Results

- FluxRETAP accurately identified gene targets whose knockdown lead to substantial increase of isoprenol titers in *P. putida*
- FluxRETAP outperformed a conventional non-computational, pathway-guided target selection
- The use of VAMMPIRE enabled accurate assembly of CRISPRi constructs containing up to five sgRNA arrays

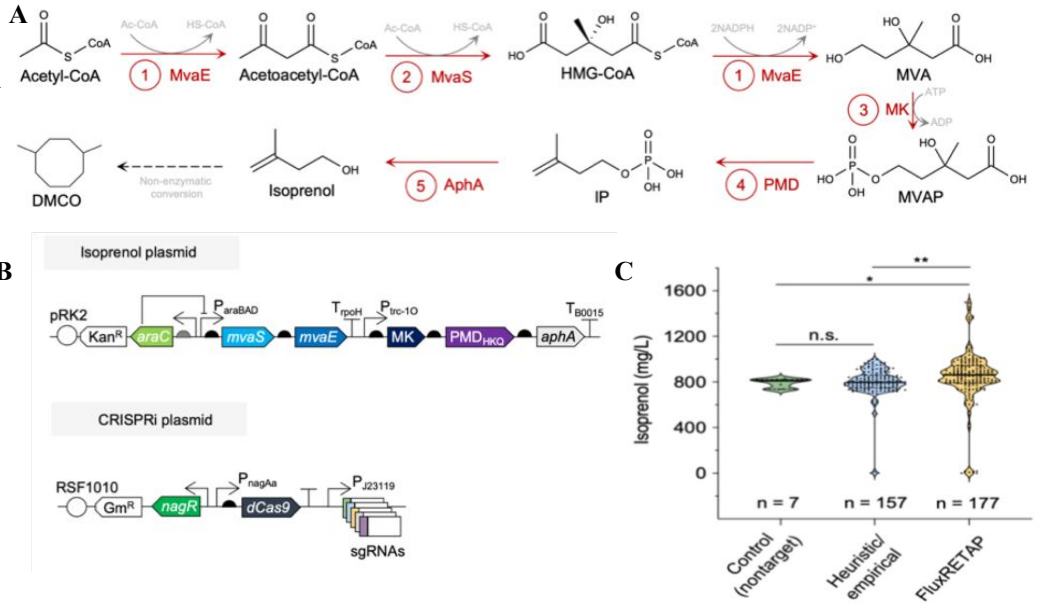

Figure caption: **(A)** Metabolic pathway for production of isoprenol. **(B)** Schematic diagram of plasmid used for isoprenol production and CRISPRi downregulation. **(C)** Comparison of isoprenol titers from intuition-based group and FluxRETAP.

Significance/Impacts

- The integration of FluxRETAP and VAMMPIRE has the potential to advance metabolic engineering and enhance bioproduction titers, with potential applicability to other microbial systems

Yunus I.S., et.al. Metab Eng. DOI: 10.1016/j.ymben.2025.11.007 (JBEI #1281)

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Advances in Ionic Liquid Recycling for Lignocellulosic Biomass Pretreatment

Background/Objective

- Biomass pretreatment is a major bottleneck due to its rigid cellulose–hemicellulose–lignin structure. Ionic liquids (ILs) can effectively deconstruct biomass but remain limited by cost and difficult recovery
- This review presents a systematic overview of the current state of research on ILs in biomass conversion with a specific emphasis on newer developments that enable their recyclability

Approach

- The review evaluates how IL structure (anion/cation chemistry) drives cellulose, hemicellulose, and lignin dissolution and assesses recent IL designs aimed at improving recyclability and reducing environmental and energy burdens
- Systematically compares advanced IL recovery methods—antisolvent systems, membranes, and distillation

Significance/Impacts

- Provides the most up-to-date assessment of recyclable ILs and practical recovery routes for biorefinery deployment
- Offers clear guidance for designing cost-effective, scalable, and environmentally aligned IL-based pretreatment systems. Supports future development of industrial biomass conversion pathways

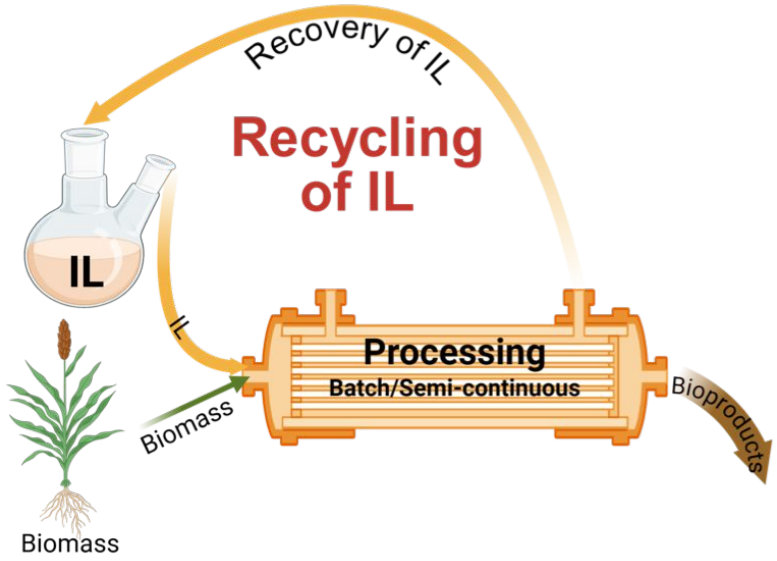

Figure caption: Conceptual overview of ionic liquid recovery recycling in lignocellulosic biomass pretreatment

Rahman M.M., et.al. Green Chemistry. DOI: 10.1039/D5GC03051J (JBEI #1282)

Engineered Accumulation of Protocatechuate in Corn Biomass to Enhance Biomanufacturing

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GREAT LAKES BIOENERGY RESEARCH CENTER

Background/Objective

- Protocatechuate (DHBA) serves as a precursor for a wide range of valuable bioproducts
- Crops can accumulate DHBA via expression of dehydroshikimate dehydratase (QsuB)
- The objective was to overproduce DHBA in plant biomass for downstream biological upgrading

Approach

- Corn was engineered to overproduce DHBA via QsuB expression. DHBA was extracted and converted into the polymer precursor 2-pyrone-4,6-dicarboxylate (PDC) using engineered *Novosphingobium aromaticivorans* (Figure 1)

Results

- Large amounts of DHBA was recovered in alkaline hydrolysates (APL) of engineered corn stover (Figure 2a)
- A 4.8-fold increase in PDC titers was achieved through biological upgrading of APL derived from QsuB corn compared to unmodified biomass (Figure 2b)

Significance/Impacts

- Our data demonstrate an strategy to valorize biomass-derived DHBA into other valuable bioproducts using engineered crops and microbial strains
- The proposed valorization of APL streams could improve the economics of advanced biofuels and bioproducts

Figure **1:** Approach for the extraction and upgrading of aromatics derived from engineered biomass overproducing DHBA.

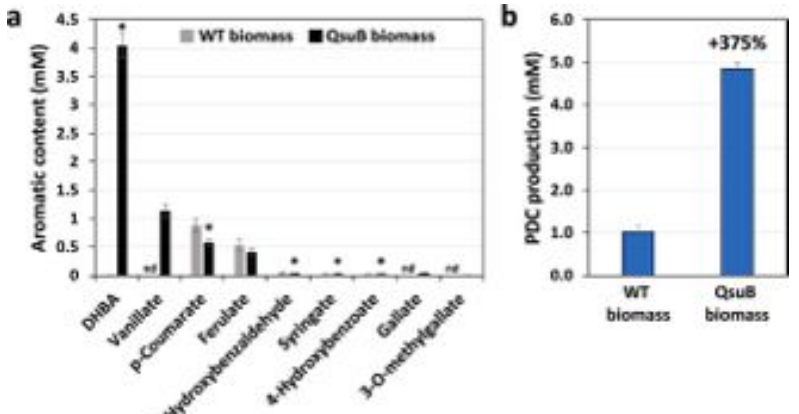

Figure **2**: APL composition and PDC production. (**a**) Aromatic content in APL obtained from wild-type (WT) and QsuB corn biomass. *$P < 0.05$. (**b**) Amount of PDC produced after growing the engineered *N. aromaticivorans* strain for 48h on APL derived from WT or QsuB corn.

Tian Y., et.al. ACS Sustain Chem Eng. DOI: 10.1021/acssuschemeng.5c09025 (JBEI #1283)

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Multiplexed profiling of transcriptional regulators in plant cells

Background/Objective

- Transcriptional regulators play key roles in plant growth, development, and environmental responses; however, understanding how their regulatory activity is encoded at the protein level has been hindered by a lack of multiplexed large–scale methods to characterize protein libraries in planta

Approach

- We present ENTRAP-seq (Enrichment of Nuclear Trans-elements Reporter Assay in Plants), a high-throughput method that introduces protein-coding libraries into plant cells to drive a nuclear magnetic sorting-based reporter, enabling multiplexed measurement of regulatory activity from thousands of protein variants

Results

- We combined ENTRAP-seq with machine-guided design to engineer the activity of an Arabidopsis transcription factor in a semi-rational fashion

Significance/Impacts

- Our findings demonstrate how scalable protein function assays deployed in planta will enable the characterization of natural and synthetic coding diversity in plants

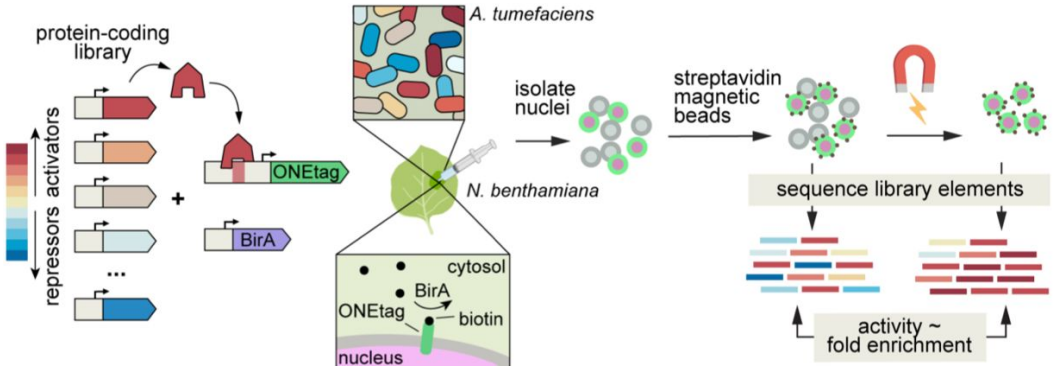

Figure caption: Overview of the ENTRAP-seq assay. A library of *trans*-acting elements, such as TFs of varying strengths, is cloned in binary vectors for *Agrobacterium*-mediated transformation. These *trans*-elements regulate a promoter driving the expression of an outer nuclear envelope tag (ONEtag) containing a biotin acceptor peptide. A transgene for the constitutive expression of the *E. coli* biotin ligase *BirA* is also included to biotinylate the ONEtag. The library as well as the ONEtag reporter and the *BirA* transgene are transformed into *Agrobacterium* and mixed cultures are infiltrated into leaves of *N. benthamiana*. The ONEtag is then biotinylated by constitutively expressed BirA using endogenous biotin. Next, nuclei are isolated from infiltrated leaves and incubated with magnetic streptavidin-coated beads, allowing for magnetic sorting. DNA is extracted from the unsorted and sorted nuclei samples and the abundance of each library element is determined by sequencing the T-DNAs. The enrichment of a given *trans*-element in the sorted versus unsorted population correlates with its activation strength.

Alamos S., et.al. Nat Biotechnol. DOI: 10.1038/s41587-025-02880-w (JBEI #1284)

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Perspectives for artificial intelligence in bioprocess automation

Background/Objective

- Recent advances in artificial intelligence (AI) have rapidly changed the lab automation landscape, promoting self-driving laboratories (SDLs) that enable autonomous scientific discovery

Approach

- This review examines three interconnected perspectives:
  - Hybrid human–machine decision-making for bioprocessing
  - Laboratory design considerations in the era of AI
  - Scale-up challenges when transitioning from screening to manufacturing

Results

- We anticipate that future SDLs will mostly take a hybrid form, in which human and AI/robotics components will intertwine not only for physical (e.g. experiments) and computational (e.g. data analysis) tasks, but also in the conception and design of studies

Significance/Impacts

- The next frontier is not the replacement of scientists but the careful design of hybrid systems, where human expertise and AI-driven automation complement one another

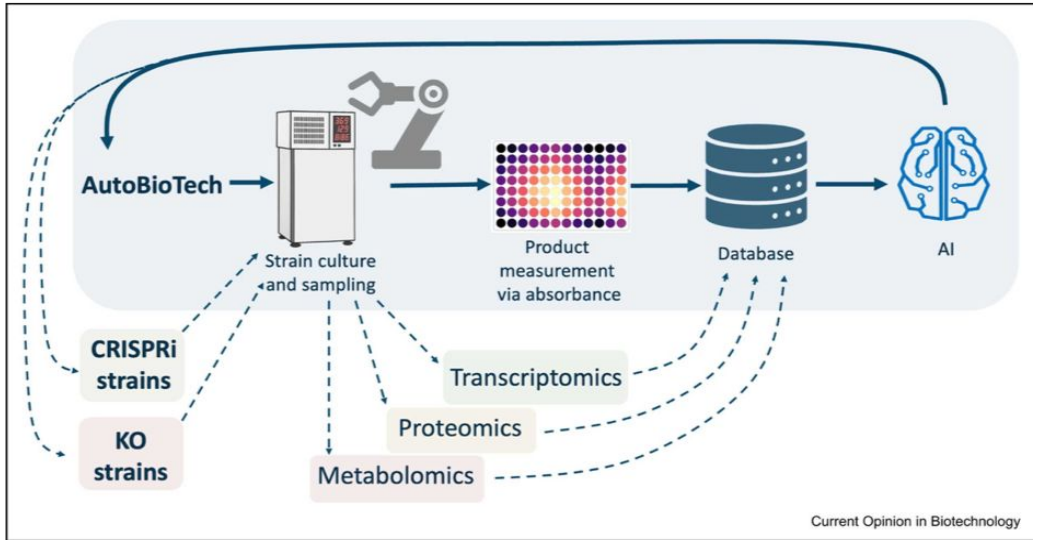

Figure caption: Illustrative example of a possible hybrid automated lab in metabolic engineering. This hybrid lab example is composed of three fully automated core processes (in blue) and five auxiliary processes (in green, orange, and red). The core processes involve the generation of strains harboring different combinatorial pathways through the use of the fully automated AutoBioTech strain construction platform, the culturing of those strains, sample acquisition, and the measuring of the final production through absorbance. Data are then stored in a database for an AI to decide how to proceed next. Attached to these three core fully automated processes, we can see several auxiliary processes (green, orange, and red) displaying different levels of automation: from highly automated (green) to some automation (orange) or purely manual (red). A second and third alternative to build strains involves producing strains with downregulated genes through CRISPRi (e.g. as in Carruthers et al. [32]), or manually knocking genes out. Alternative ways to phenotype the strain involve transcriptomics, proteomics or metabolomics. These data need not be collected in every single DBTL cycle, but could, through multifidelity approaches, improve the quality of the active learning processes to design new strains.

Helleckes L.M., et.al. Curr Opin Biotechnol. DOI: 10.1016/j.copbio.2025.103392 (JBEI #1285)

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Biomanufacturing from gaseous C1 feedstocks: A perspective on opportunities and challenges

Background/Objective

- Explore opportunities and challenges in biomanufacturing using gaseous C1 feedstocks for sustainable production of fuels and chemicals

Approach

- Analyzes existing infrastructure and research on biomanufacturing from gaseous C1 feedstocks and identified areas for improvement
- Reviews microbial and bioelectrochemical conversion methods, including both aerobic and anaerobic processes

Results

- Highlights the potential of CO_2, CO, and CH_4 as C1 feedstocks for biomanufacturing fuels and chemicals
- Compares the use of pure versus mixed cultures for targeted bioproduct generation
- Identifies major barriers to commercialization, such as strain engineering, bioprocess, and safety challenges
- Recommends research tools and safety measures to enable efficient, safe, and scalable C1 biomanufacturing

Significance/Impacts

- Supports domestic biomanufacturing capacity and supply chain resilience.
- Enhances biosecurity and mitigates potential risks

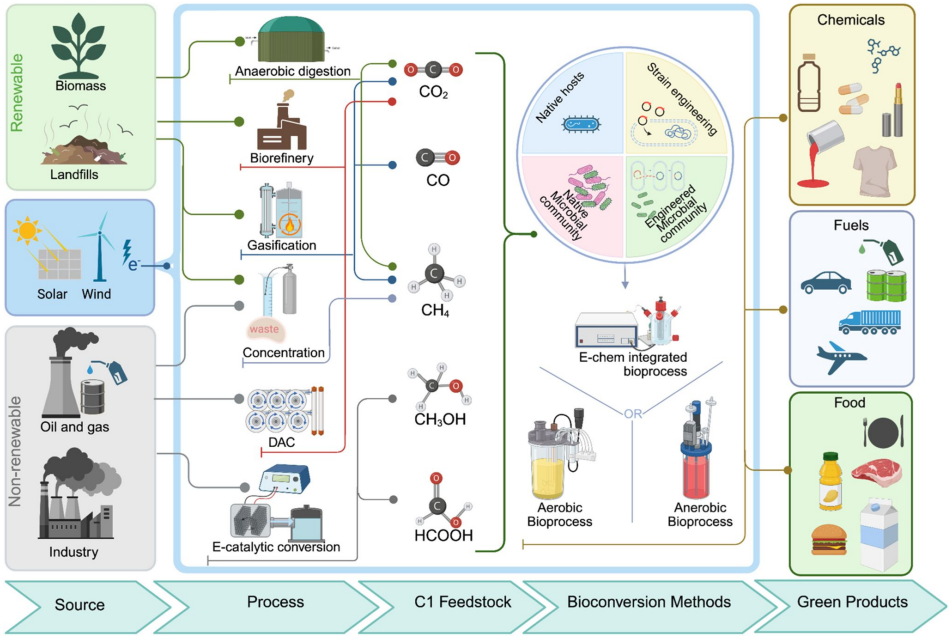

Figure caption: Illustration of a future economy based on C1 biomanufacturing.

Rachbauer L., et.al. Energy Nexus. DOI: 10.1016/j.nexus.2025.100601 (JBEI #1286)

Enabled Publications

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Integrated Biorefinery of Brewer's Spent Grain for Second-Generation Ethanol, Mycoprotein, and Bioactive Vinasse Production

Background/Objective

- Brewer's spent grain (BSG), the main by-product of brewing, is underutilized. This work develops an integrated biorefinery to convert BSG into ethanol, fungal biomass, and bioactive vinasse

Approach

- Evaluated biological + Deep Eutectic Solvent (DESs) pretreatment strategies for improved sugar accessibility
- Conducted one-pot saccharification/fermentation for 2G-ethanol production and assessed its vinasse as a plant biostimulant

Results

- Best ethanol yield: 0.46 g/g (≈89% theoretical) with [Ch]Cl:lactic acid DES
- Vinasse from same DES pretreatment improved tomato growth metrics

Significance/Impacts

- Enables multi-product valorization from a single waste stream
- Supports circular economy and sustainable brewery waste management

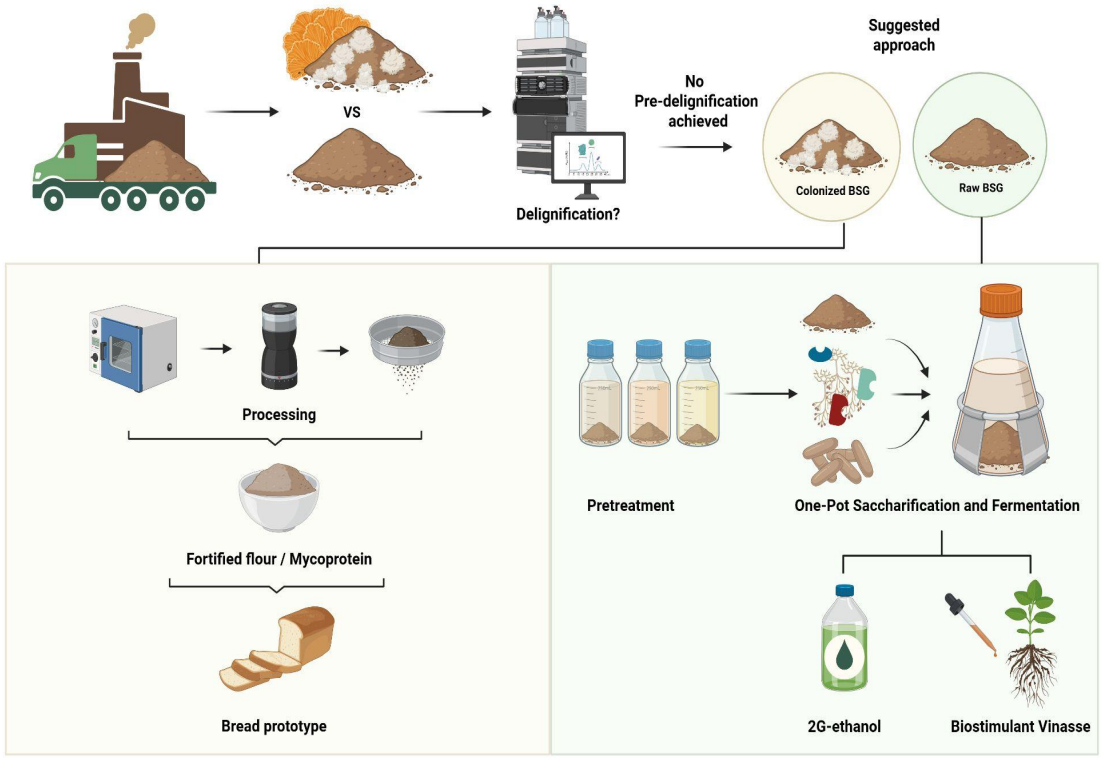

Figure caption: Proposed biorefinery routes transform brewer's spent grain into fortified food, 2G ethanol, and biostimulant vinasse.

Saldarriaga-Hernandez S., et.al. Fermentation. DOI: 10.3390/fermentation11110627 (JBEI #126)

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Accelerating Gene Discovery with KitBase: Rapid Identification of the *DHD6* Flowering Pathway

Background/Objective

- Heading date is a critical trait determining rice yield and regional adaptability, controlled by complex genetic networks.
- Identify key gene mutated in early flowering mutants..

Approach

- Screened KitBase to isolate early-flowering mutants. Identified candidate gene as DHD6.
- Validated gene function using CRISPR-Cas9 KO and genetic complementation assays

Results

- *DHD6* encodes a WD40-domain protein; its loss-of-function leads to early flowering caused by the upregulation of the florigen promoter *Ehd1*.
- *DHD6* acts synergistically with *Se14* and *PHYC* to delay flowering.

Significance/Impacts

- Identifies *DHD6* as a key negative regulator, providing new alleles that can be used in breeding programs to optimize plant adaptability to different latitudes.
- Establishes a "fast-cycling" triple mutant model (*dhd6 phyC se14*) with a significantly shorter life cycle, which accelerates future functional genomics research in rice and sorghum.

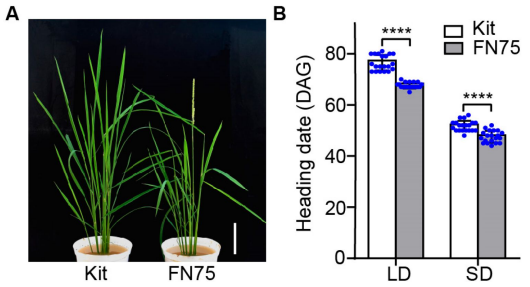

Figure 1. (A) Line FN75 and the control line Kitaake (Kit). (B) Heading dates of Kitaake and FN75 under LD and natural short-day conditions during winter.

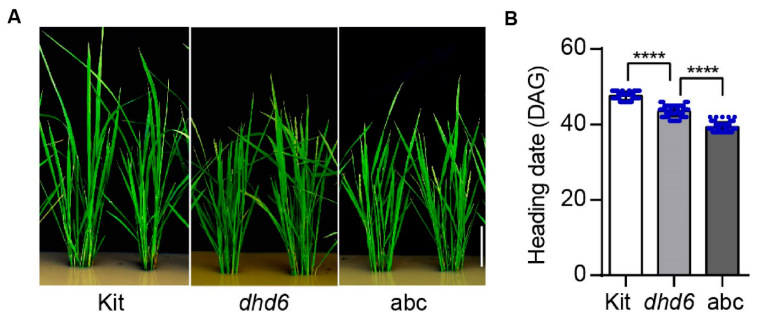

Figure 2. (A) Kitaake, dhd6, and the dhd6 phyC se14 triple mutant line abc at the heading stage in the field. (B) Heading date of Kitaake, *dhd6*, and the *dhd6 phyC* se14 triple mutant line abc.

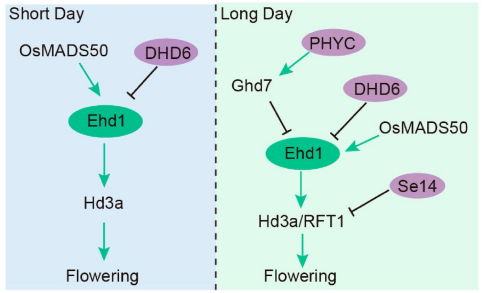

Figure 3. Model of DHD6 and other flowering-time genes regulating flowering under long-day and short-day conditions.

Sun, Q.; et al. *Plants* 2025, 14, 3503. https://doi.org/10.3390/plants14223503 (JBEI #127)