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Enhancement of sorghum biomass hydrolysis by addition of amylases to an ionic liquid-based deconstruction process

Background/Objective

- In addition to lignocellulose, forage and biomass sorghum varieties can also contain significant amounts of non-fibrous carbohydrates like starch that should be considered for conversion to biofuels and bioproducts.

Approach

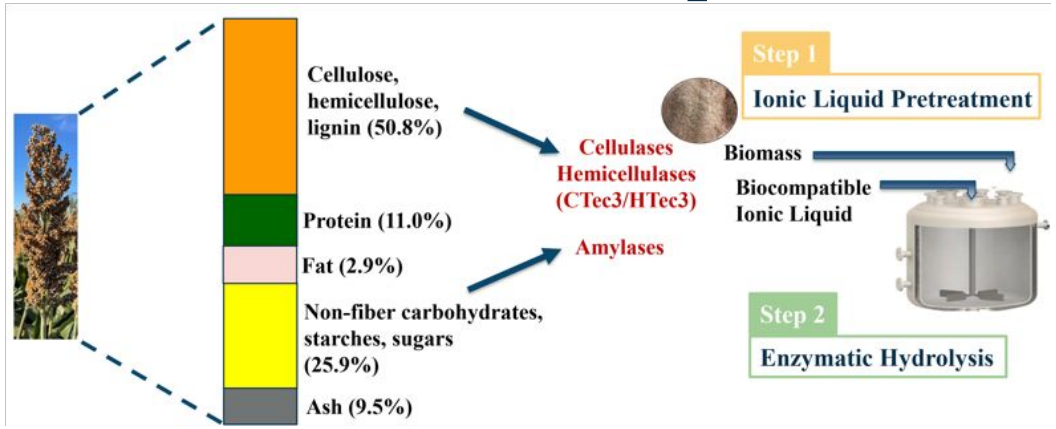
- We report a method to maximize the enzymatic hydrolysis of whole plant samples containing fibrous and non-fibrous carbohydrates. This process involves the addition of amylases to ionic liquid-pretreated biomass in conjunction with cellulase and hemicellulase cocktails.

Results

- We found that amylases are compatible with cholinium-based ionic liquids and act synergistically with other saccharolytic enzymes to deconstruct sorghum biomass and release up to 153 g/L of monomeric sugars.
- By using mixtures of sorghum grain (high starch content) and stover (low starch content), we show that amylases can produce up to 4-fold higher glucose yields from biomass with a higher starch content but even samples containing only stover can benefit from amylase addition.

Significance/Impacts

- This study presents new possibilities for process intensification during the deconstruction of starch-containing bioenergy feedstocks.



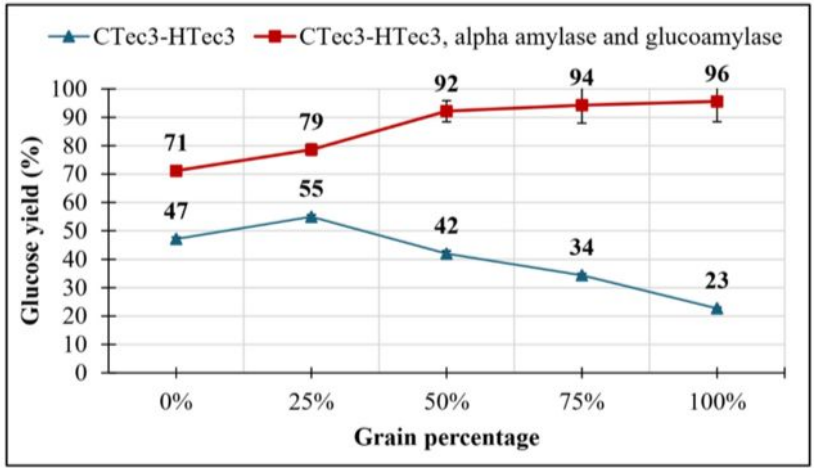


Figure caption: Top: Scheme of the simultaneous conversion of fibrous and non-fibrous carbohydrates in sorghum to fermentable sugars. Bottom: Glucose yields obtained from different grain and stover mixtures after cholinium phosphate pretreatment and enzymatic hydrolysis using cocktails containing only cellulase and hemicellulase (CTec3-HTec3) or cellulase, hemicellulase, alpha-amylase and glucoamylase enzymes

Islam, M.T., et.al. BMC Res Notes. doi: 10.1186/s13104-025-07469-9 (JBEI#1287)

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A "Sweet" Biorefinery: Sugar-Derived Ionic Liquids for the Pretreatment of Lignocellulosic Biomass

Background/Objective

- High-performance ionic liquids (ILs) are typically derived from petroleum resources. Furthermore, conventional IL pretreatments necessitate water washing and pH adjustment.
- To develop sugar-derived ILs that realize a self-reliant biorefinery by eliminating inefficient post-pretreatment steps.

Approach

- Cholinium- and betainium-based ILs were synthesized using biomass-derived cations and sugar-derived anions.
- A streamlined one-pot process was implemented and pretreatment efficacy on sorghum and pine was evaluated.

Results

- The feasibility of process simplification was validated by achieving high sugar yields of Cholinium gluconate ([Ch][GlcA]) via one-pot process (Figure 2).
- The biocompatibility of Betainium gluconate ([Bet][GlcA]) was confirmed through the direct bioconversion of pretreated hydrolysates into bisabolene (Figure 3).

Significance/Impacts

- The one-pot process significantly reduces capital and operational expenditures by eliminating auxiliary steps while maintaining high pretreatment efficacy.
- A self-reliant biorefinery maximizes resource efficiency and minimizes waste.

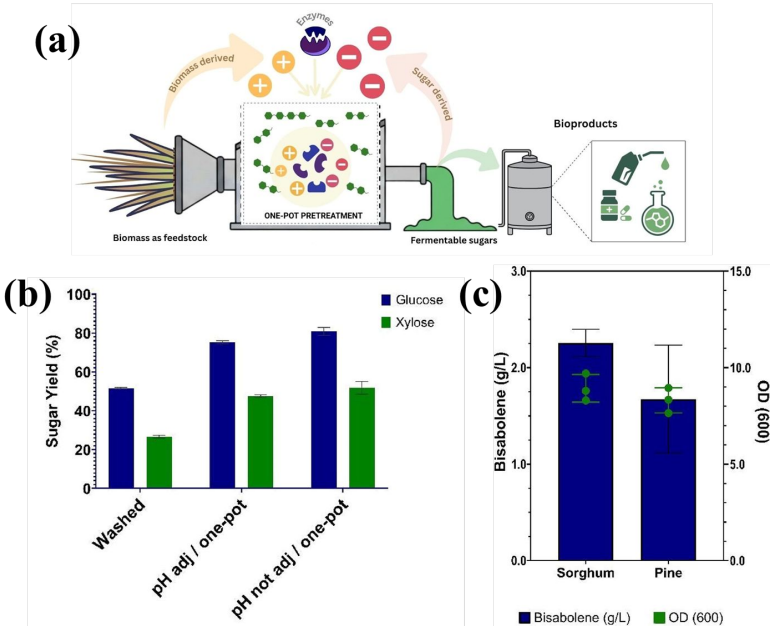

Figure caption: (a) Overview of a "sweet" biorefinery based on sugar-derived ILs. (b) Impact of post-pretreatment steps on the efficacy of [Ch][GlcA] for sorghum pretreatment. (c) Bisabolene production and cell growth of *R. toruloides* in biomass hydrolysates pretreated with [Bet][GlcA].

Kim, M., et.al. Journal of Ionic Liquids. doi: 10.1016/j.jil.2025.100184 (JBEI#1288)

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A highly active Burkholderia polyketoacyl-CoA thiolase for production of triacetic acid lactone

Background/Objective

- Triacetic acid lactone (TAL) is a renewable polyketide platform chemical, but microbial production is limited by inefficient acetyl-CoA condensation.
- This work aimed to identify a high-activity polyketoacyl-CoA thiolase to enable carbon-efficient TAL biosynthesis.

Approach

- 2000+ thiolases were mined and screened, identifying BktB from *Burkholderia* as a high-performance TAL producer.
- BktB was characterized using *in vivo* TAL production, *in vitro* enzyme kinetics, X-ray crystallography, and structure-guided engineering.

Results

- BktB significantly increased TAL titers and productivity compared to prior TAL producing enzymes.
- Structural analysis revealed active-site features underlying enhanced activity and enabled further enzyme improvement.

Significance/Impacts

- BktB clarifies the structure-function-mechanism in microbial polyketide biosynthesis, advancing TAL as a scalable bio-based platform molecule.
- This enzyme framework broadly supports carbon-efficient biosynthesis of polyketide-derived chemicals aligned with JBEI's mission.

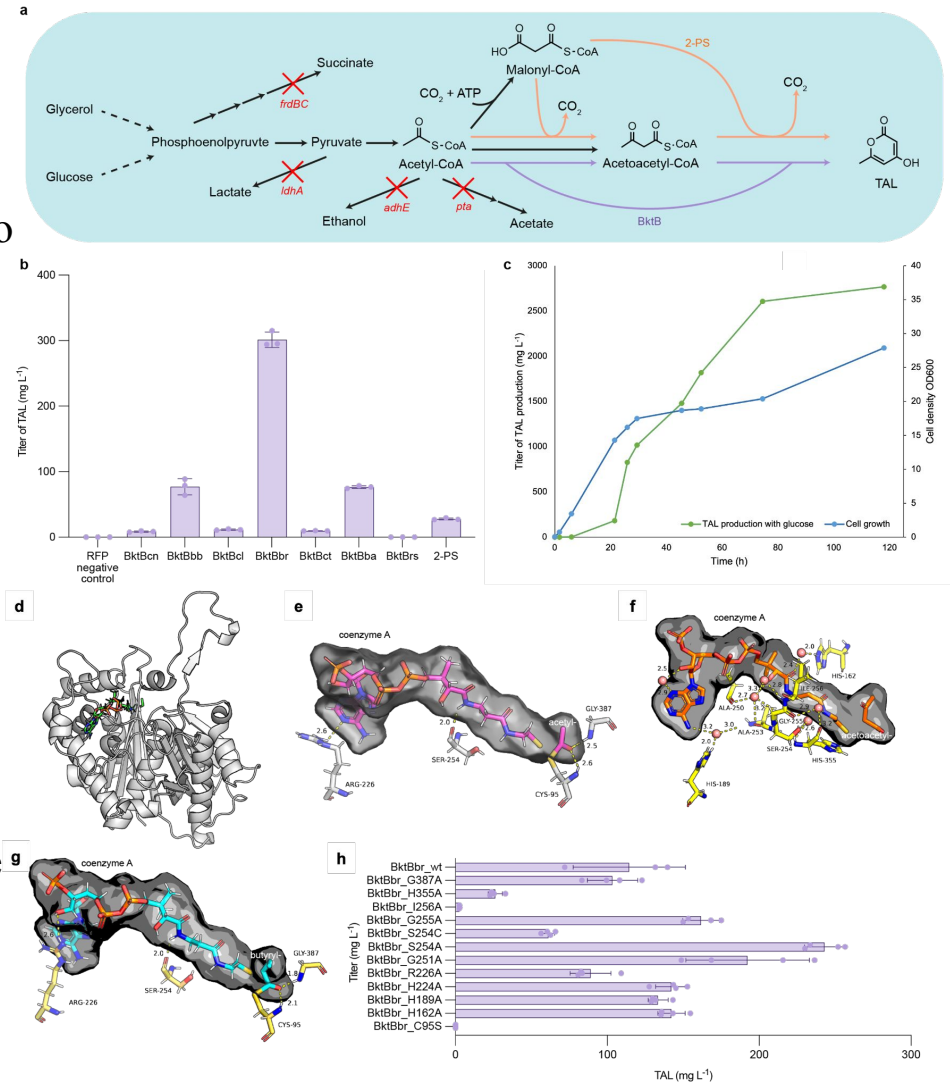

Figure caption: *In vivo* TAL production by BktB from *Burkholderia*. a) Pathway scheme of TAL production in engineered *E. coli*. Comparison of BktB pathway (purple) and 2-PS pathway (orange) were shown. b) TAL titers from different BktBs and 2-PS. c) 2.8 g/L TAL titers from bioreactor cultivation. d) 2 Å X-Ray structure of BktBbr. e-g) Co-crystallization structure of BktBbr with e) acetyl-, f) acetoacetyl, and g) butyryl- group. h) Engineered BktBbr further increased the enzyme activity.

Wang, Z., et.al. Nature communications. doi: 10.1038/s41467-025-65946-y (JBEI#1289)

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Automation and machine learning drive rapid optimization of isoprenol production in Pseudomonas putida

Background/Objective

- Microbial bioproduction is limited by complex metabolic networks and vast combinatorial design spaces.
- This study aimed to optimize isoprenol production in *Pseudomonas putida* using automated experimentation guided by machine learning.

Approach

- An automated Design-Build-Test-Learn pipeline combined CRISPRi-based gene perturbations with active machine learning.
- High-throughput proteomics was used to validate gene repression and inform model predictions.

Results

- Machine-learning-guided optimization achieved an approximately five-fold increase in isoprenol production.
- The approach efficiently reduced a large genetic design space to a small set of high-impact strain designs.

Significance/Impacts

- This work demonstrates that machine learning and automation can substantially accelerate metabolic engineering.
- The framework is broadly applicable to optimizing diverse microbial hosts and bioproducts.

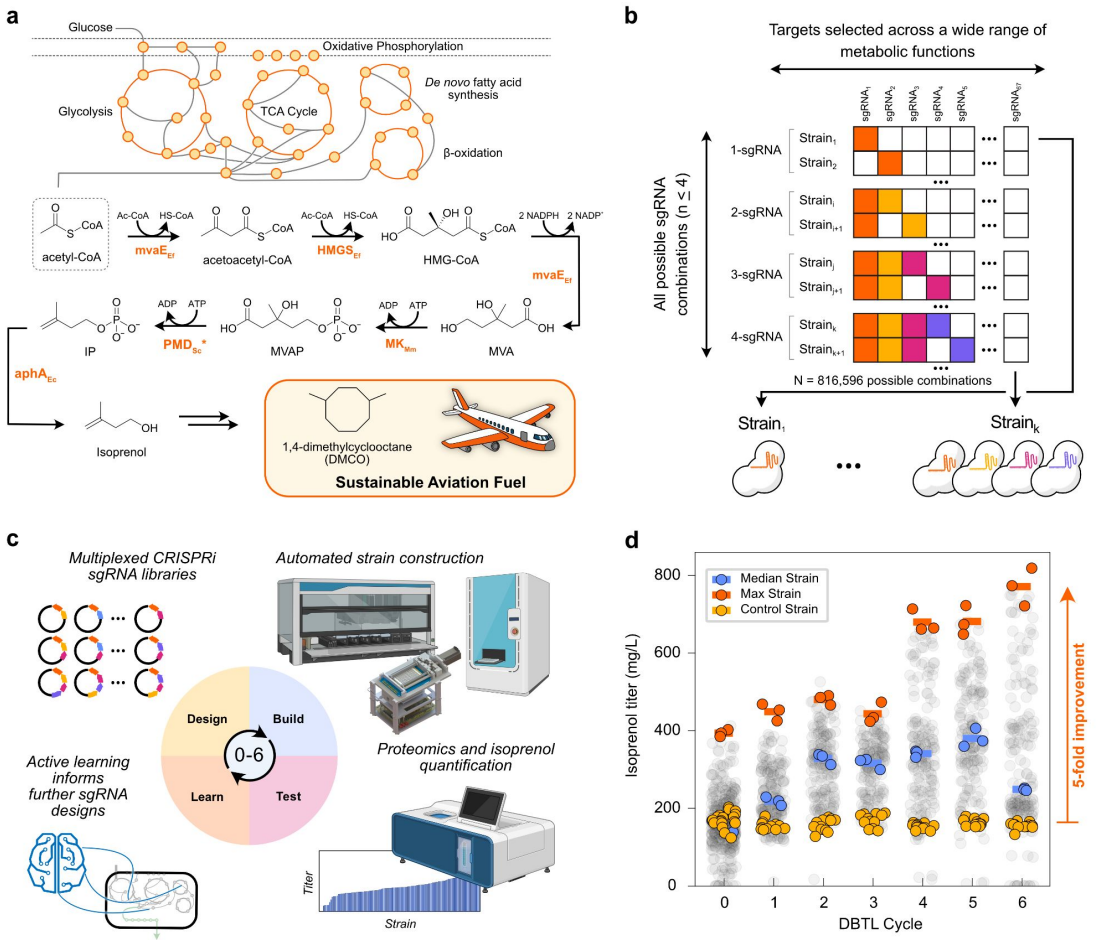

Figure caption: Machine-learning-guided optimization of isoprenol production in *Pseudomonas putida*. Combinatorial CRISPRi gene downregulation, was explored using an automated Design-Build-Test-Learn pipeline. High-throughput strain construction enabled efficient navigation of a large sgRNA design space, resulting in a five-fold increase in isoprenol titer over six optimization cycles.

Carruthers, D.N., et.al. Nature communications. doi: 10.1038/s41467-025-66304-8 (JBEI#1290)

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A Simple and Versatile Cell-Free Expression Method for Producing Secondary Metabolites

Background/Objective

- Secondary metabolites are a major source of natural products with industrially relevant bioactivities.
- Lysate-based cell-free expression is an emerging platform for accelerating the discovery and engineering of these natural products.

Approach

- We developed a generalizable and simple set of reaction conditions that support high-yield protein expression in lysates derived from *Streptomyces venezuelae* NRRL B-65422 and *Streptomyces lividans* TK24.

Results

- These extracts enable iterative and pathway-level biosynthesis, as demonstrated by the production of the polyketide flaviolin and the cyclic dipeptide albonoursin.
- The *S. lividans* lysate outperforms the *E. coli* systems by also supporting the expression and catalytic activity of a (~250kDa) type I polyketide synthase (T1PKS), producing its corresponding product.

Significance/Impacts

- This represents the first demonstration coupling both expression and catalysis of a megasynthase in a Streptomyces-based system, and of a T1PKS in any bacterial extract.

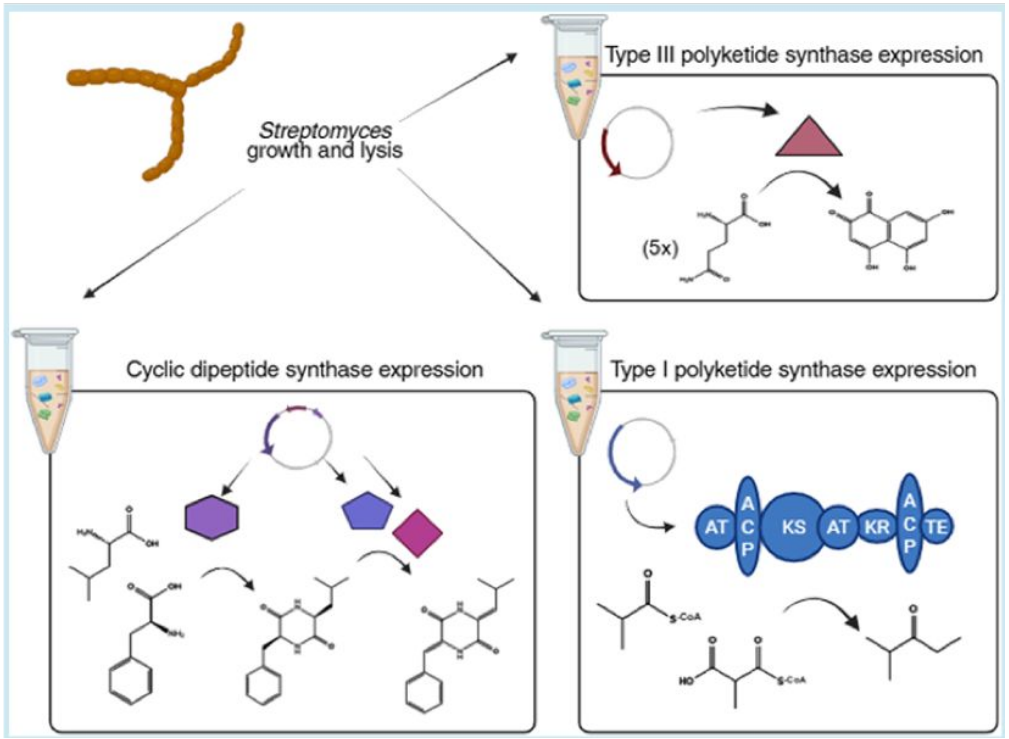

Figure caption: A simple and versatile cell-free expression method for producing secondary metabolites

Dinglasan, J.L.N., et.al. ACS synthetic biology. doi: 10.1021/acssynbio.5c00497

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Generating functional plasmid origins with OriGen

Background/Objective

- Generative AI aids biological design, but no prior system could create sequences capable of autonomous replication.
- Train a language model (OriGen) on plasmid replicons to generate novel, functional origins of replication.

Approach

- Trained a deep learning model on a comprehensive database of plasmid replicons and host associations.
- Synthesized the predicted sequences and experimentally validated their replication function in E. coli.

Results

- OriGen achieved 75% prediction accuracy, outperforming general genomic models like Evo.
- Generated origins proved functional in vivo despite having low sequence identity to natural plasmids.

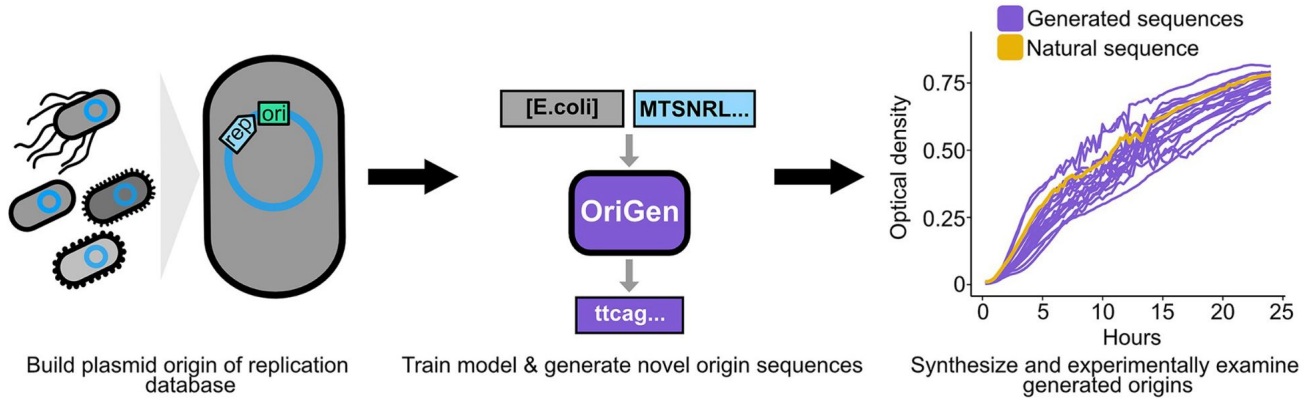

Figure caption: This graphical abstract illustrates a three-step workflow where a plasmid origin of replication database is built, used to train the OriGen model to generate novel sequences, and finally validated through the experimental synthesis and examination of those origins.

Significance/Impacts

- Demonstrates the first AI-generated biological sequences capable of autonomous replication.
- Enables the rapid generation of bespoke vectors and exploration of novel sequence space.

Irvine, Jamie, et.al. Nucleic Acids Research. doi.org/10.1093/nar/gkaf1198 (JBEI#1292)

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Identification of proteins influencing CRISPR-associated transposases for enhanced genome editing

Background/Objective

- CRISPR-associated transposases (CASTs) allow large payload integration but suffer from low efficiency.
- Objective: Identify host factors that influence CAST activity to improve bacterial genome editing.

Approach

- Performed a genome-wide loss-of-function screen in *E. coli* to uncover proteins affecting transposition.
- Engineered strains with identified factors (e.g., lambda-Red system) to test efficiency gains.

Results

- Identified 15 host factors; utilizing lambda-Red improved editing efficiency by 55.2-fold in *E. coli*.
- Successfully translated these improvements to other species like *P. putida* and *K. michiganensis*.

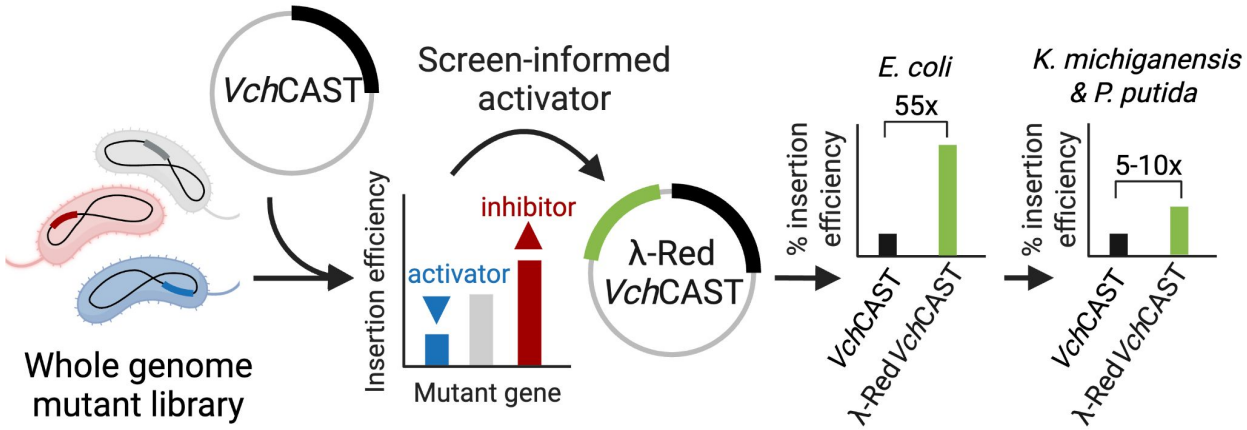

Figure caption: Schematic of the RB-TnSeq screen to identify E. coli genes affecting VchCAST integration efficiency.

Significance/Impacts

- Overcomes major bottlenecks to transform CASTs into robust tools for precise bacterial editing.
- Validates a strategy of manipulating host factors to optimize genetic tools in non-model organisms.

Song, L.C.T., et.al. Science Advances. doi.org/10.1126/sciadv.aea1429 (JBEI#1293)

Enabled Publications

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Techno-economic assessment-guided biofoundry for microbial strain development

Background/Objective

- A biofoundry integrates laboratory automation with DBTL workflows to accelerate strain development for sustainable manufacturing.
- Quantifying the economic efficiency of automated processes remains challenging.

Approach

- We define the robot-assisted module (RAM) as a plug-and-play unit for constructing workflow and apply the Experiment Price Index (EPI) to evaluate and optimize synthetic biology workflows.

Results

- Using EPI calculation and RAMs, we developed four workflows for strain development: guide (g)RNA cloning, genome editing, DNA assembly, and sample analysis.
- We further extended the EPI framework on techno-economic assessment (TEA) by estimating return on investment (ROI) and payback periods for bio foundry operations at varying project scales.

Significance/Impacts

- EPI is effective for experimental planning, scalable biofoundry deployment, and serves as a universal tool for evaluating automation efficiency.

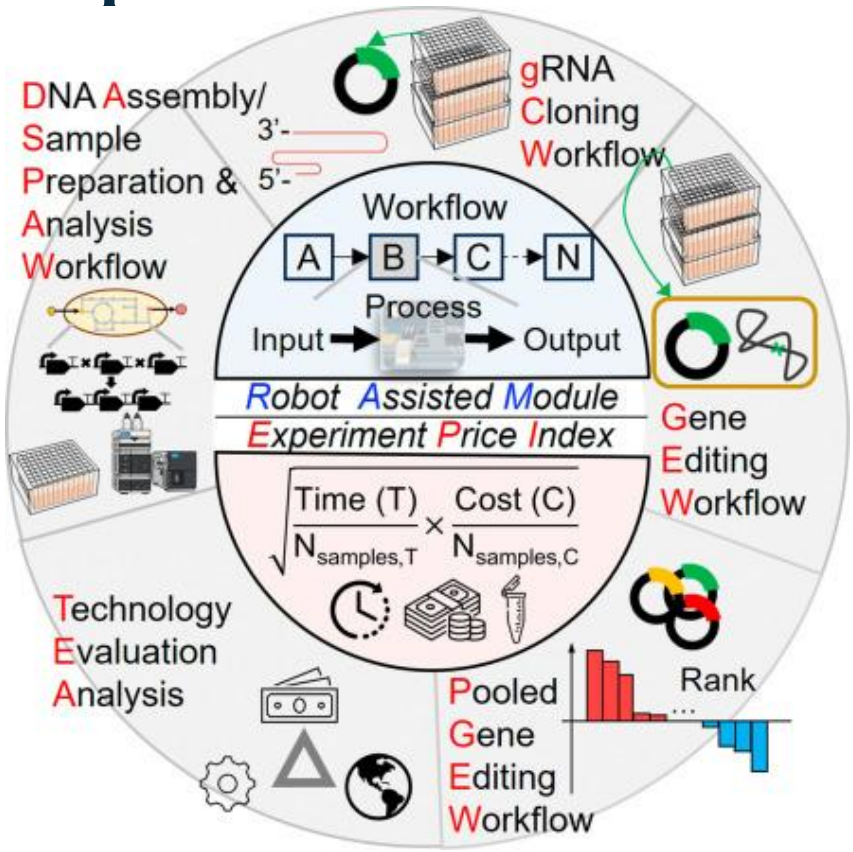

Figure caption: A robotics-assisted biofoundry platform was applied to synthetic biology, using robot-assisted module (RAM)-based work flows optimized with the Experiment Price Index (EPI). Demonstrated through the development of biofoundry workflows, the EPI framework was further extended to techno-economic analysis for evaluating annual biofoundry operations.

Heo, Y.B., et.al. Trends in biotechnology. doi: 10.1016/j.tibtech.2025.11.002 (JBEI#129)

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Multi-Omics Analyses Reveal Divergent Molecular Mechanisms Underlying Plant Biomass Conversion by Five Fungi

Background/Objective

- Filamentous fungi use distinct strategies for plant biomass deconstruction, but mechanisms underlying divergence remains unresolved.
- Compare the molecular mechanisms underlying plant biomass degradation across different fungal strains

Approach

- We used a multi-omic analysis of fungi grown on defined plant biomass substrates to capture condition-specific regulatory and metabolic responses.
- Used systems biology and bioinformatics tools to integrate data

Results

- Identified strain-specific transcriptional programs and regulatory factors controlling
- Revealed divergent metabolite and protein expression patterns linked to differences in carbohydrate catabolism and cofactor metabolism

Significance/Impacts

- We developed a framework for engineering of fungal cell factories for improved bioconversion of lignocellulosic feedstocks.
- Improved set of criteria for selection and optimization of fungal strains for industrial biomass processing

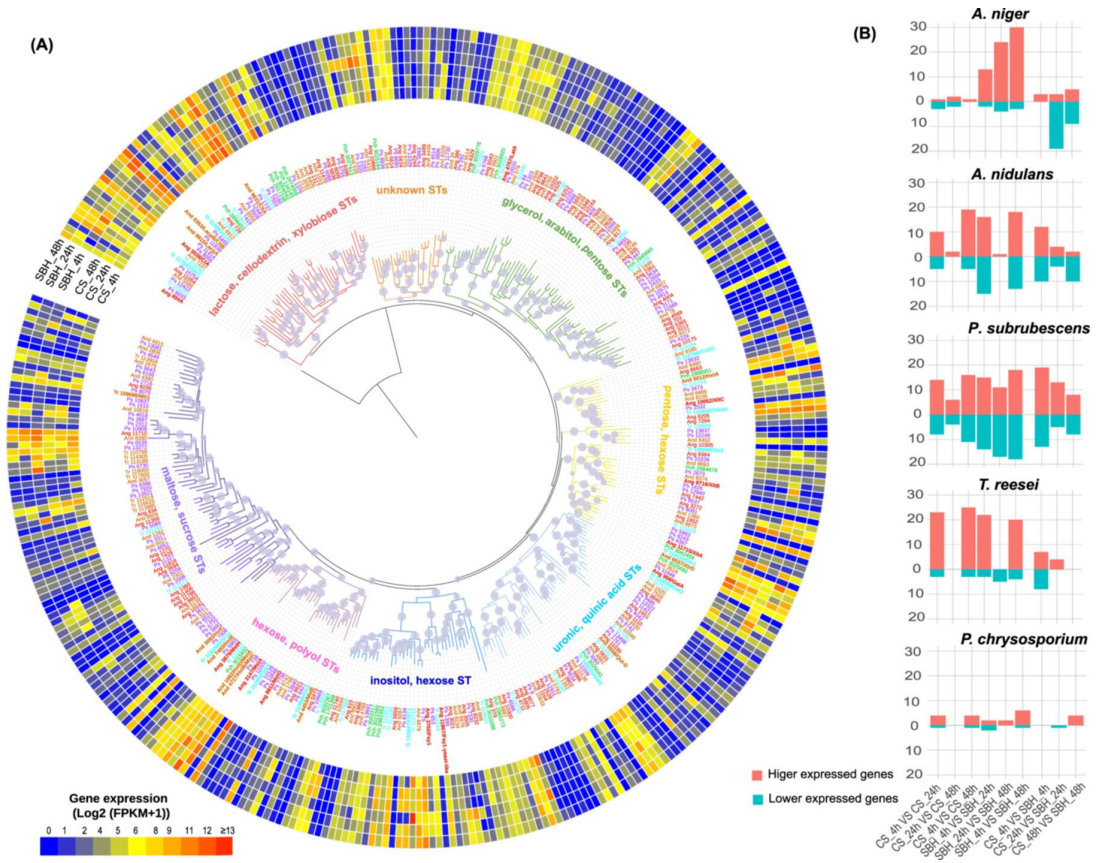

Figure caption: Detailed transcriptome profile of sugar transport (ST) genes in five fungi. (A) Expression and phylogenetic profiles of ST genes from five species. Expression levels from low to high are indicated by blue to red.(B) Total number of significantly higher and lower expressed ST genes in each comparison, shown in red and cyan, respectively.

Peng, M., et.al. MicrobiologyOpen. doi: 10.1002/mbo3.70201 (JBEI#130)

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Enzymology and Structural Basis of Glycosyltransferases Involved in Saponin C28 Carboxylic Acid O-D-Fucosylation

Background/Objective

- QS-7/QS-21 are glycosylated triterpene (saponin) adjuvants that potentiate adaptive immune response in vaccination
- Altering the identity/linkages of sugars can enhance potency or physicochemical properties, but requires enzymological characterization of enzymes that perform tehse modifications

Approach

- Fucosyltransferases from *Q. saponaria* (QS-7/21) and *S. vaccaria* were purified and characterized in vitro for substrate scope/kinetic studies
- Both enzymes were also crystallized and subjected to X-ray diffraction

Results

- The first sugar of the core pharmacophore was rapidly diversified in vitro, providing access to new saponins and QS-7/QS-21 intermediates
- X-ray crystallography was used to solve the first structures of 4-keto-6-deoxy-D-glucosyltransferases

Significance/Impacts

- This study provides a springboard for producing new-to-nature, glycodiversified analogs of QS-7/QS-21 towards creating an arsenal of designer adjuvants optimized for specific antigens/vaccine formulations
- Crystal structures will enable modulating substrate scope to enable more effective biocatalytic production of these analogs

Hudson, G.A., et.al. JACS Au. doi: 10.1021/jacsau.5c00907 (JBEI#131)

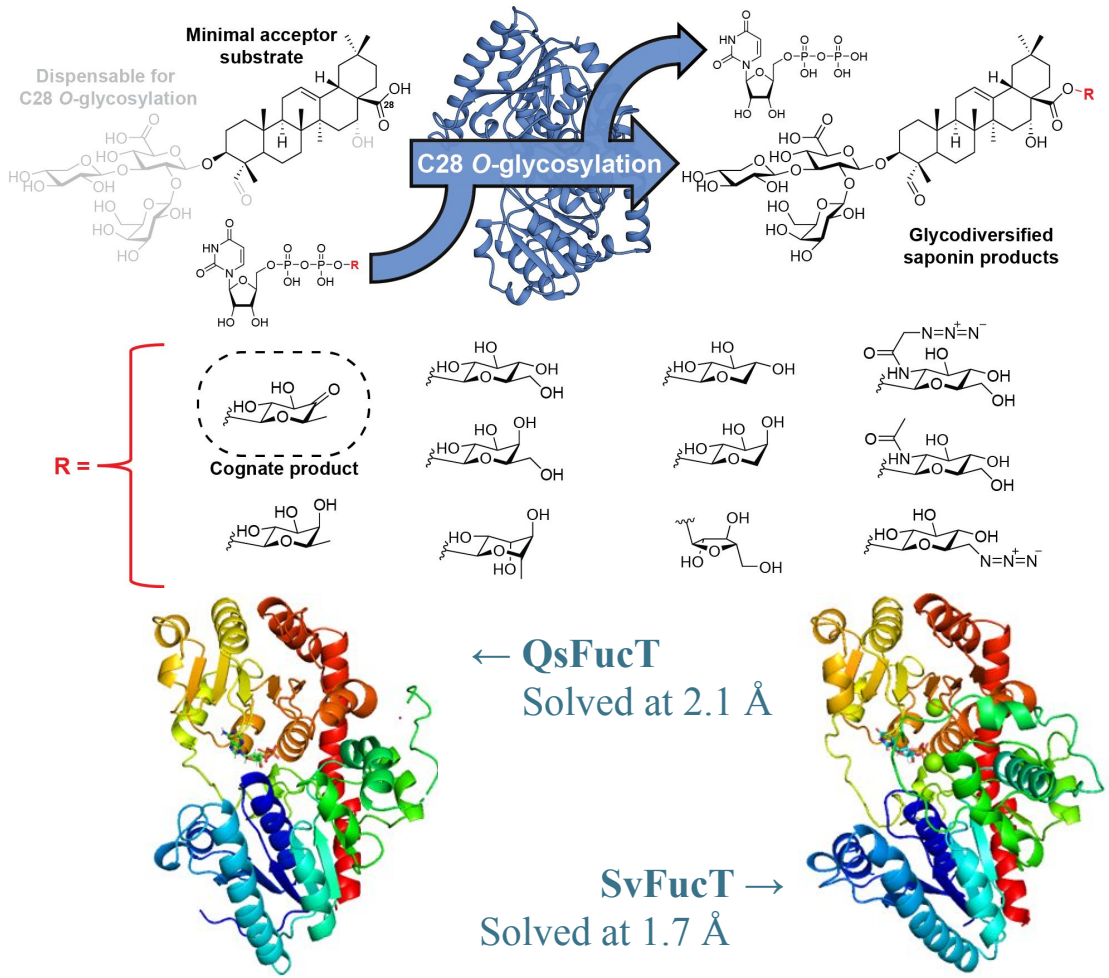

Figure caption: The first sugar of the QS-21/QS-7 core pharmacophore, natively D-Fuc, was rapidly diversified in vitro using purified enzymes. These enzymes were also crystsallized, leading to the founding crystallographic structures of 4-keto-6-deoxy-D-glucosyltransferases