TridentSynth: A Webtool for the Retrosynthesis of Molecules using Chimeric Type I Polyketide Synthases and Chemoenzymatic Pathways

Background/Objective

- Most retrosynthesis tools cover only chemistry or monofunctional enzymes, leaving multifunctional PKSs out of reach.
- Build a web platform that unifies type I PKS, monofunctional enzymes, and synthetic chemistry for pathway design.

Approach

- Deployed a Django web app coupling RetroTide PKS design with DORAnet enzymatic and chemical pathway search.
- Integrated DORA-XGB ML feasibility scores and a reaction database for enzyme retrieval and ranking.

Results

- TridentSynth proposed PKS-enzyme routes several valuable molecules.
- Platform returns top closely matched natural reactions per step with UniProt links enzymatic steps for experimental follow-up.

Significance/Impacts

- Advances DOE BER goals for predictive design of sustainable bio-based routes to fuels and chemicals.
- Free webtool at tridentsynth.lbl.gov accelerates JBEI bioeconomy pipelines from target molecule to lab pathway.

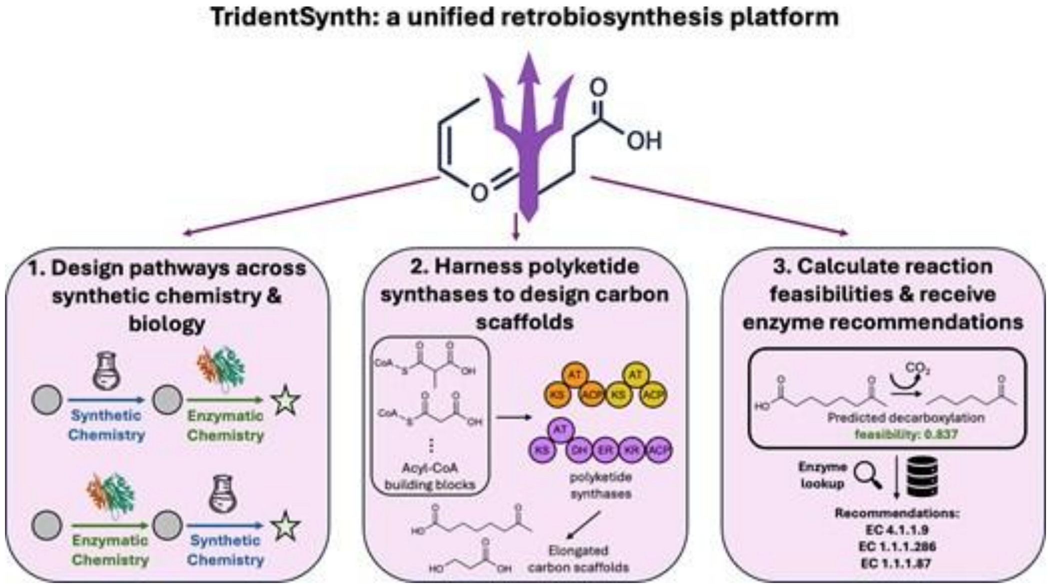

Figure caption: (1) Design pathways across synthetic chemistry & biology: Integrates synthetic organic, monofunctional enzymatic, and multifunctional enzyme logic to map hybrid pathways. (2) Harness polyketide synthases to design carbon scaffolds: Programs Type I PKSs to iteratively condense acyl-CoA building blocks into diverse carbon backbones. (3) Calculate reaction feasibilities & receive enzyme recommendations: Generates post-PKS steps via DORAnet, using machine learning to score step feasibility and querying a curated database to recommend matching candidate enzymes.

Chainani, Y., et.al. Nucleic Acids Research. doi: 10.1093/nar/gkag471 (JBEI #1313)

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Demonstrating a Butylamine-based Deconstruction Method for Poplar Biomass and Conversion by Diverse Microbial Strains

Background/Objective

- Lignocellulosic biomass needs scalable pretreatment, but most solvents are costly, toxic, or hard to recover.
- Test butylamine pretreatment of poplar and biocompatibility of hydrolysates with three industrial microbes.

Approach

- Pilot-scale butylamine pretreatment of poplar followed by enzymatic hydrolysis and LC-MS toxicity profiling.
- Charcoal detoxification then fermentation with *A. niger*, *P. putida* and *R. toruloides* for bioproducts.

Results

- Detoxified hydrolysate contained >100 g/L sugars; charcoal cut butylacetamide by 94% to 0.4 g/L from 7.1 g/L.
- Hydrolysate supported malic acid, isoprenol, and bisabolene production, outperforming defined media.

Significance/Impacts

- Advances DOE BER mission for low-impact biomass deconstruction with recyclable solvents and microbial conversion.
- Supports JBEI bioenergy pipeline by linking poplar pretreatment to scalable production of fuel and chemical targets.

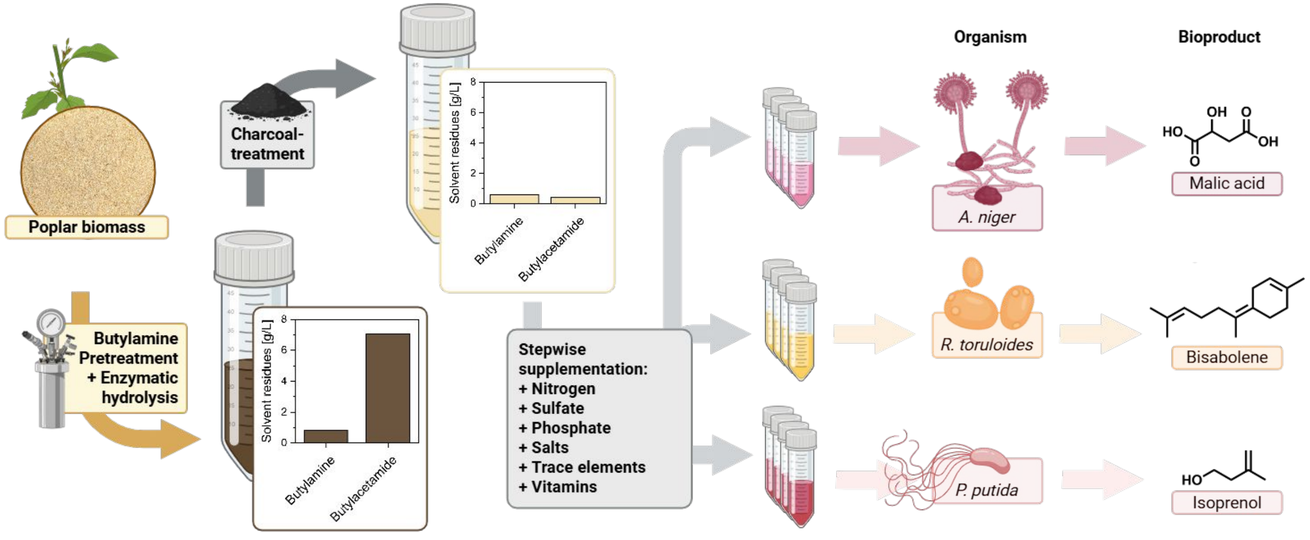

Figure caption: Pretreatment of poplar biomass with butylamine released > 100 g/L of fermentable sugars and supported the biosynthesis of three different bioproducts.

Jia, J., et.al. Green Chemistry. doi: 10.1039/d5gc06335c (JBEI #1314)

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ClURGT4 Contributes to Biomass Accumulation in Watermelon by Influencing Cell Size and Cell Wall Composition

Background/Objective

- Genes that control fruit size and biomass via cell wall sugar transport remain poorly defined in crop plants.
- Characterize ClURGT4, a putative Golgi UDP-Rha/UDP-Gal transporter, for its role in watermelon biomass accumulation.

Approach

- Generated *clurgt4* loss-of-function mutants in *Citrullus lanatus* and analyzed cell wall sugars and morphology.
- Used proteomics and glycosylation profiling to identify downstream cell wall metabolism changes.

Results

- ClURGT4 had UDP-Gal/UDP-Rha transport activity in vitro.
- Mutants showed reduced biomass and lower Gal, Rha, and GalA in cell walls, with smaller cells from reduced expansion.
- Proteomic data linked altered protein glycosylation to cell wall metabolism proteins in *clurgt4* mutants.

Significance/Impacts

- Aligns with DOE BER goals to understand plant cell wall biosynthesis underpinning sustainable bioenergy crops.
- Informs JBEI feedstock engineering by revealing levers for biomass accumulation through nucleotide sugar transport.

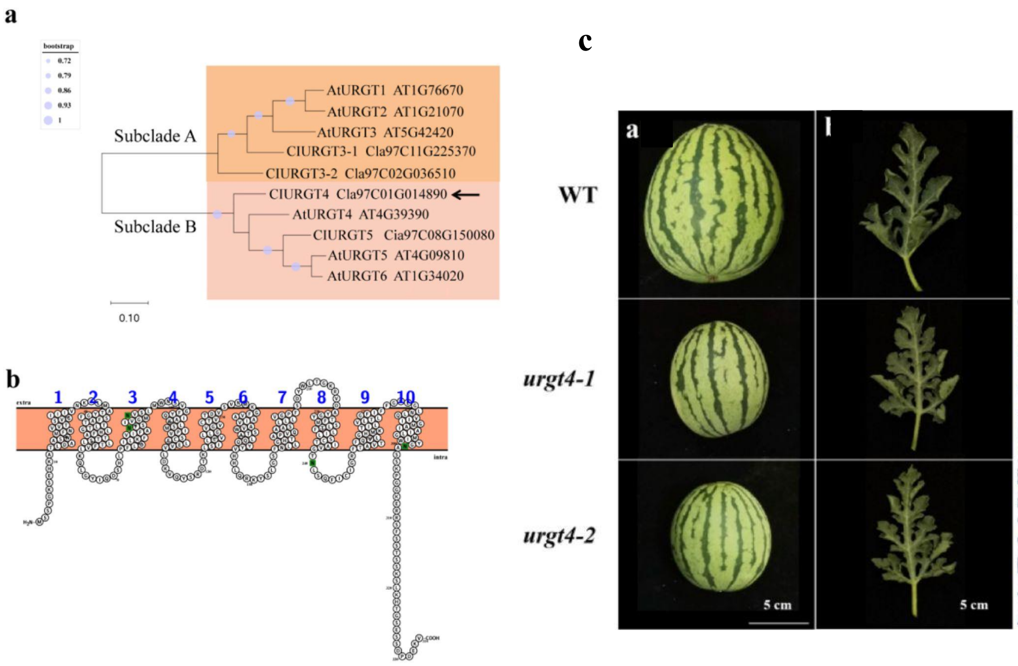

Figure caption: Fig. 1. Identification and analysis of ClURGTs in watermelon. (**a**) A neighbor-joining phylogenetic tree of *Arabidopsis* (At) and watermelon (Cl, *C. lanatus*) URGTs. ClURGT4 is marked by a black arrow in subclade B. (**b**) Analysis of ClURGT4 transmembrane domains. (**c**) Fruits and leaves were smaller in *clurgt4* mutants demonstrating the role of ClURGT4 in biomass accumulation

Gao, G., et.al. Agriculture Communications. doi: 10.1016/j.agrcom.2026.100149 (JBEI #1315)

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Expanding the Scope of Redox-balance Growth Coupling Techniques with a Carbon Co-feeding Strategy

Background/Objective

- Redox-balance growth-coupled selections usually require feeding the reductase substrate, blocking acyl-CoA pathways.
- Extend growth coupling to acetyl-CoA-derived reduction pathways via a dual-feedstock co-feeding strategy.

Approach

- Engineered NADPH-imbalanced E. coli and co-fed glucose with acetate or propionate as a redox-neutral carbon source.
- Applied selection to evolve a class II HMG-CoA reductase from Delftia acidovorans toward NADPH use.

Results

- Acetate co-feeding linked acetaldehyde, 3-HB, and mevalonate production linearly to growth in selection strains.
- Selection enriched HMGR variants with improved NADPH activity; propionate covered acyl-chain elongation.

Significance/Impacts

- Supports DOE BER goals to engineer microbes for sustainable production of fuels and chemicals from renewable carbon.
- Broadens JBEI's directed-evolution toolkit for polyketide, isoprenoid, and fatty-acid bioproduction pathways.

Cowan, A., et.al. Metabolic Engineering. doi: 10.1016/j.ymben.2026.05.004 (JBEI #1316)

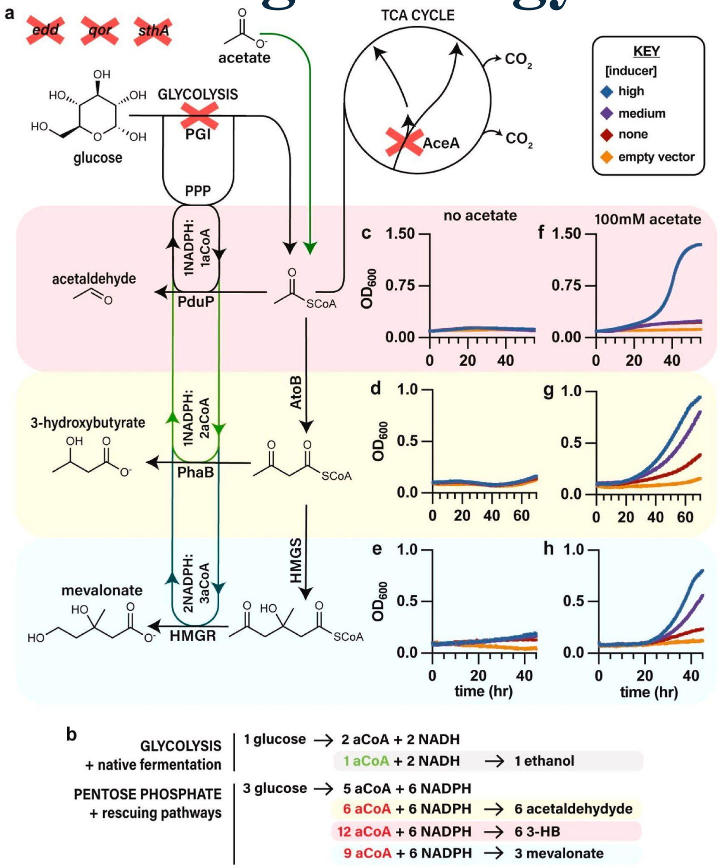

Figure caption: Fig. 1. Acetate co-feeding enables growth coupling of partially reduced acyl-CoA pathways. (a) APEQS was engineered to accumulate NADPH and prevent growth on acetate, while expressing pathways to acetaldehyde, 3-HB, or mevalonate. (b) Stoichiometry shows that these partially reducing pathways require more acetyl-CoA than glucose alone can provide under redox-balanced conditions. (c–e) Without acetate, pathway expression does not rescue growth. (f–h) With 100 mM acetate, growth is rescued in an induction-dependent manner. Cultures were grown in MOPS medium with 2% glucose, with or without acetate and IPTG induction; n = 3.

Diol-enhanced Natural Deep Eutectic Solvents for Efficient Poplar Pretreatment

Background/Objective

- Natural deep eutectic solvents (DES) are promising for biomass pretreatment but suffer from high viscosity at scale.
- Develop ternary DES (TDES) with diols to cut viscosity and improve poplar fractionation.

Approach

- Formulated ChCl/3,4-dihydroxybenzoic acid (DHBA) TDES with 1,4-butanediol (1,4-BDO) or ethylene glycol; tested viscosity and lignin solubility.
- Pretreated wild-type and DHBA-enriched transgenic poplar; ran enzymatic digestibility and lignin structure assays.

Results

- Diol TDES stayed liquid at room temperature, raised lignin solubility, and preserved β-O-4 linkages vs binary DES.
- TDES improved delignification and enzymatic digestibility; 1,4-BDO TDES worked on engineered DHBA-rich poplar.

Significance/Impacts

- Advances DOE BER goals for sustainable lignocellulose conversion using recyclable, low-toxicity solvents.
- Couples with JBEI's engineered poplar lines to enable integrated bioproduct and high-value lignin streams.

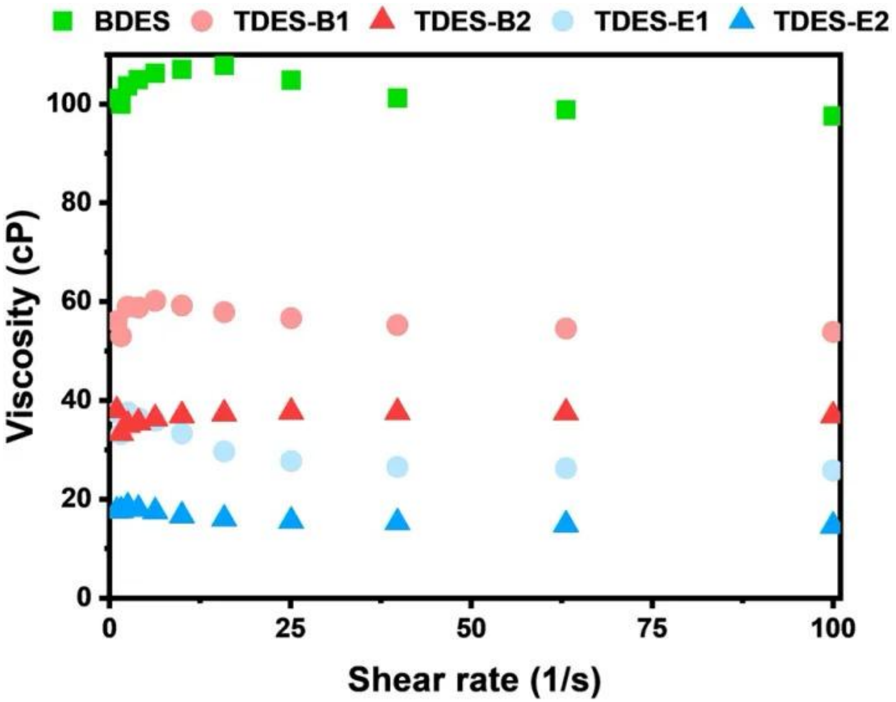

Figure caption: Presents the viscosity behavior of ChCl-DHBA DESs measured at 140 °C, corresponding to the biomass pretreatment temperature in this study. BDES exhibited a relatively high viscosity of 98 cP at 100 s^{-1}, while the viscosities of TDESs were significantly decreased: TDES-B1 (54 cP) >TDES-B2 (37 cP) >TDES-E1 (26 cP) >TDES-E2 (15 cP). The results indicate that the introduction of both diols significantly reduced the viscosity of BDES, improving solvent fluidity and mass transfer characteristics.

Yoon, C., et.al. Frontiers in Chemistry. doi: 10.3389/fchem.2026.1790752 (JBEI #1317)

Cost and Carbon Implications of Flexible Biomanufacturing to Produce Bio-based Gasoline, Jet Fuel, and Platform Chemicals

Background/Objective

- Single-product biorefineries face price volatility and limited flexibility to respond to market shifts.
- Assess cost and CO_2 emissions trade-offs of a multiproduct sorghum biorefinery covering fuels and chemicals.

Approach

- Developed a techno-economic model of a multiproduct biorefinery converting ensiled biomass into a suite of products, including carboxylic acids, isoprenol, DMCO, and indigoidine.
- Performed scenario analyses across different product portfolios, recovery rates, and selling prices.

Results

- Multiproduct allocation strongly governs economics, with net profits exceeding ~$111–$123 per bone-dry ton under favorable product mixes.
- Nearly 46.8% lignin diversion to indigoidine is needed to achieve parity with 2020 isoprenol prices, while 17.2% is required under 2050 price projections.

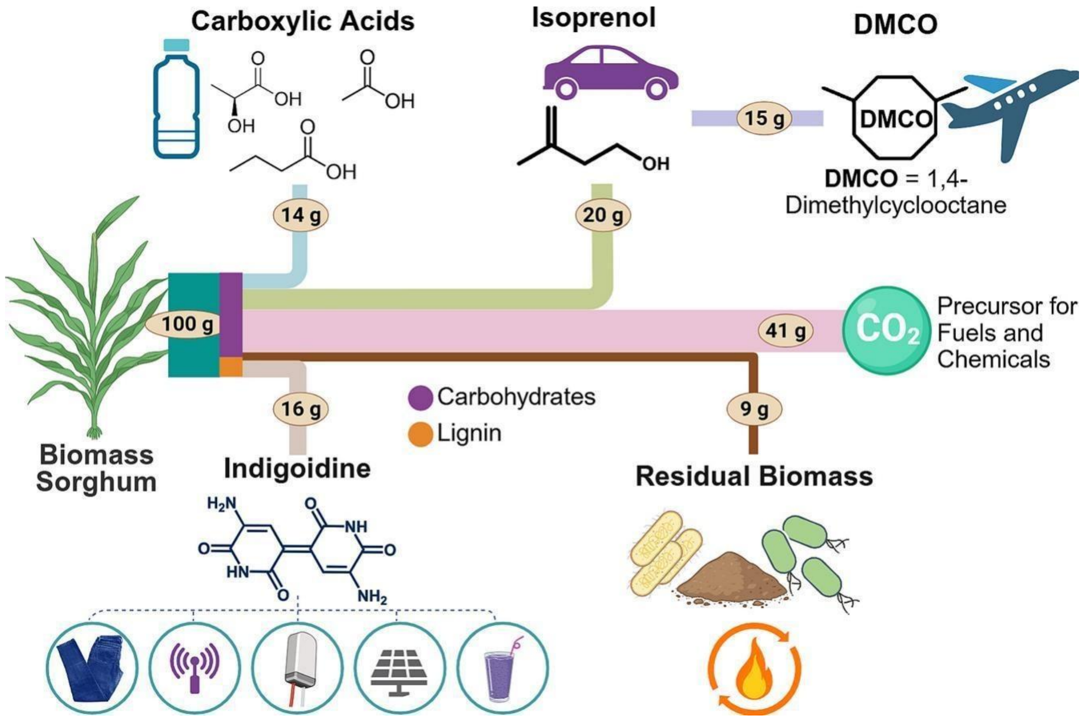

Figure caption: Overview of a multiproduct biorefinery workflow using ensiled biomass sorghum, illustrating a portfolio-based production strategy that converts biomass sorghum into a flexible suite of biobased products, including platform chemicals (carboxylic acids), gasoline and jet fuel blendstocks (isoprenol and DMCO), and high-value specialty precursors (indigoidine).

Significance/Impacts

- Advances DOE BER goals for affordable biorefinery designs that improve domestic supply resilience.
- Supports JBEI bioeconomy roadmap by quantifying flexible product portfolios for sorghum-based platforms.

Baral, N., et.al. Chemical Engineering Journal. doi: 10.1016/j.cej.2026.177641 (JBEI #1318)

Polyketide Synthase-based Controlled Synthesis of Polycyclopropanated Fuel Molecules

Background/Objective

- Aviation and heavy transport need biofuels with high energy density and low freezing points to cut CO_2 emissions.
- Engineer an iterative PKS to control chain length and cyclopropanation of POP-FA biofuel candidates.

Approach

- Computed fuel properties of POP-FAs and surveyed natural POP biosynthesis homologs across Streptomyces hosts.
- Used in vivo gene exchange of cyclopropanase domains to retune chain length and saturation.

Results

- Made shortened POP-FAs with better predicted freezing points and fully cyclopropane-saturated higher-energy variants.
- Cyclopropanase catalysis was identified as the key control point for POP-FA structural engineering.

Significance/Impacts

- Supports DOE BER mission to deliver sustainable, energy-dense biofuels for hard-to-electrify transport sectors.
- Strengthens JBEI's PKS platform for designer fuels from lignocellulosic carbon, advancing aviation decarbonization.

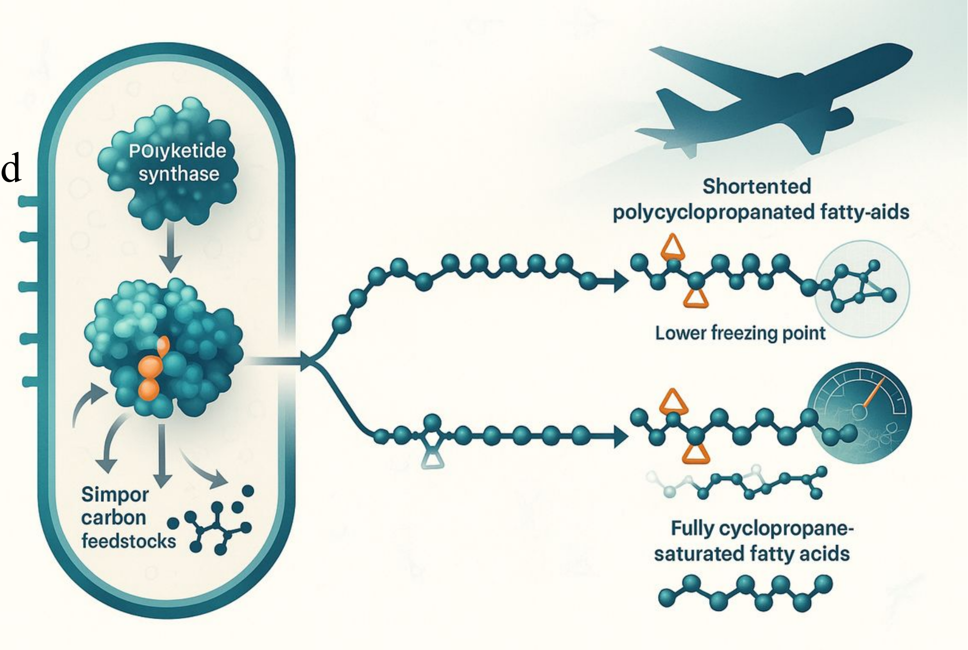

Figure caption: Engineered polyketide synthase (iPKS) pathways for the controlled synthesis of structural variants of high-energy POP fuels.

Yin, K., et.al. Nature Communications. doi: 10.1038/s41467-026-73172-3 (JBEI #1319)

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Exploring the Potential of *Rhodosporidium toruloides* in Biomanufacturing: A Comprehensive Review

Background/Objective

- Rhodosporidium toruloides is an emerging non-conventional yeast host gaining significant attention in synthetic biology and metabolic engineering
- We reviewed genetic tools for Rhodosporidium toruloides as a chassis for industrial-scale biomanufacturing.

Approach

- Surveyed Agrobacterium-mediated transformation (ATMT), CRISPR/Cas-based editing, and counter-selection strategies.
- Used isoprenoid biosynthesis engineering as a case study to expose chassis advantages and engineering bottlenecks.

Results

- We provided a comprehensive summary of the current progress in genetic tool development for R. toruloides
- We found key bottlenecks: low transformation efficiency, scarce parts, poor HR, and no stable plasmid systems.

Significance/Impacts

- Supports DOE BER goals to advance non-conventional microbial chassis for sustainable biomanufacturing.
- Strengthens the isoprenoid biofuel and lignocellulose conversion programs with a versatile yeast chassis.

Goswami, S., et.al. Frontiers in Synthetic Biology. doi: 10.3389/fsybi.2026.1796791 (JBEI #1320)

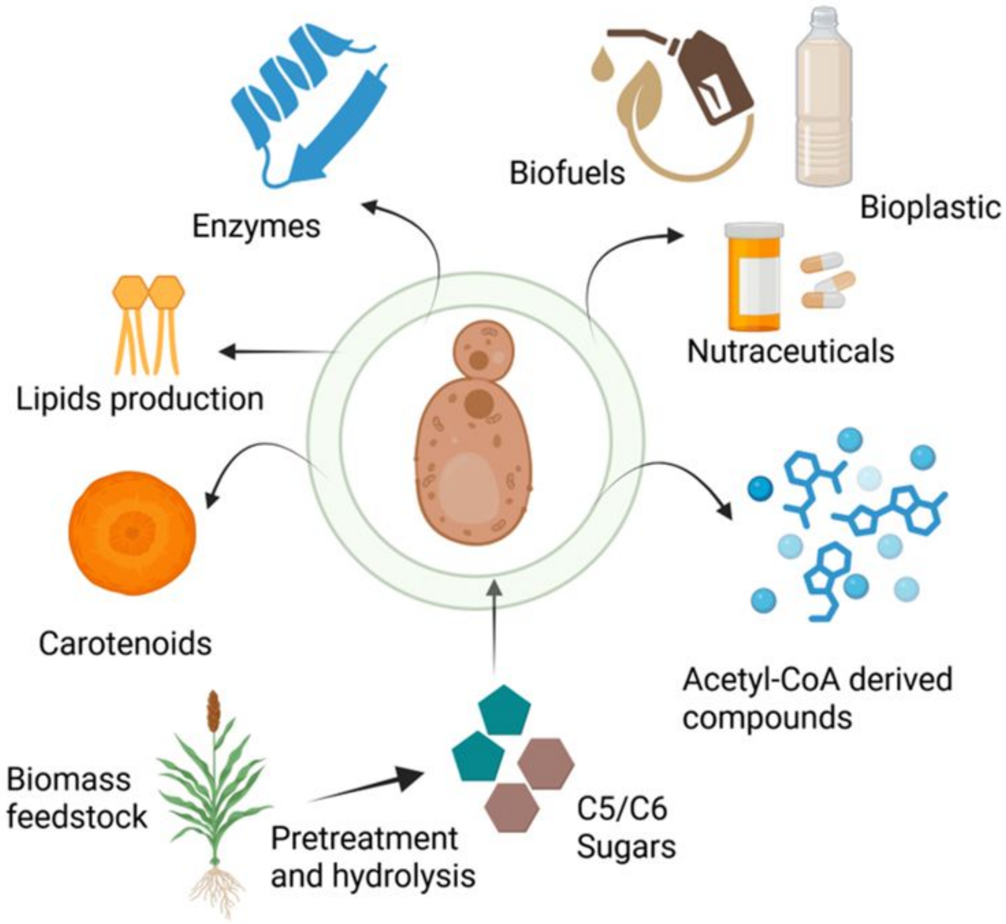

Figure caption: R. toruloides as a host for the production of wide variety of compounds and biomaterials using plant biomass as feedstocks. R. toruloides can tolerate wide range of inhibitors from pretreated biomass and is known for lipid and carotenoid production

Enabled Publications: N/A